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(54) Title: METHODS AND COMPOSITIONS FOR MODULATING RESPONSIVENESS TO CORTICOSTEROIDS

(57) Abstract: Method for modulating responsiveness to corticosteroids in a subject are provided. In the method of the invention, an agent which antagonizes a target that regulates production of IFN-γ in the subject is administered to the subject in combination with a corticosteroid such that responsiveness of the subject to the corticosteroid is modulated as compared to when a corticosteroid alone is administered to the subject. In one embodiment, the agent is an IL-18 antagonist. In another embodiment, the agent is an interleukin-12 (IL-12) antagonist. In yet another embodiment, the agent is an NK cell antagonist. In a preferred embodiment, the agent is an inhibitor of a caspase family protease, preferably an ICE inhibitor. In another preferred embodiment, the agent is an anti-IL-12 monoclonal antibody. In yet another preferred embodiment, the agent is an anti-asialo-GM1 antibody or an NK1.1 antibody. Other preferred agents include phosphodiesterase IV inhibitors and beta-2 agonists. The methods of the invention can be used in the treatment of a variety of inflammatory and immunological diseases and disorders. Pharmaceutical compositions comprising an agent which antagonizes a target that regulates production of IFN-γ in a subject, a corticosteroid and a pharmaceutically acceptable carrier are also provided. A preferred composition comprises an ICE inhibitor, a corticosteroid and a pharmaceuticaly acceptable carrier.

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METHODS AND COMPOSITIONS FOR MODULATING RESPONSIVENESS TO CORTICOSTEROIDS

Related Applications

This application is a continuation-in-part of PCT Application No. PCT/US98/04916, designating the United States, filed on March 12, 1998, which was a continuation-in-part of U.S. Patent Application Serial No. 09/016,346, filed on January 30, 1998, now abandoned, which was a continuation-in-part of U.S. Patent Application Serial No. 08/820,692, filed on March 18, 1997, now allowed. The entire contents of each of the aforementioned applications are expressly incorporated herein by reference.

Background of the Invention

Standard therapy for a variety of immune and inflammatory disorders includes administration of corticosteroids, which have the ability to suppress immunologic and inflammatory responses. Corticosteroids are used in the treatment of disorders such as asthma, autoimmune diseases (*e.g.*, rheumatoid arthritis, systemic lupus erythematosus) and transplant rejection (for reviews on corticosteroids, see *e.g.*, Truhan, A.P. *et al.* (1989) *Annals of Allergy* 62:375-391; Baxter, J.D. (1992) *Hospital Practice* 27:111-134; Kimberly, R.P. (1992) *Curr. Opin. Rheumatol.* 4:325-331; Weisman, M.H. (1995) *Curr. Opin. Rheumatol.* 7:183-190). Corticosteroids are also used topically in the treatment of various dermatological disorders, such as contact dermatitis, psoriasis vulgaris, lichen planus, keloids and urticaria pigmentosa (for a review, see Sterry, W. (1992) *Arch. Dermatol. Res.* 284 (Suppl.):S27-S29).

While therapeutically beneficial, the use of corticosteroids is associated with a number of side effects, ranging from mild to possibly life threatening. Complications associated with prolonged and/or high dose steroid usage include musculoskeletal effects (*e.g.*, osteoporosis, myopathy, aseptic necrosis of bone), ophthalmic effects (*e.g.*, posterior subcapsular cataracts), gastrointestinal effects (*e.g.*, ulcers, pancreatitis, nausea, vomiting), cardiovascular effects (*e.g.*, hypertension, atherosclerosis), central nervous system effects (*e.g.*, pseudotumor cerebri, psychiatric reactions), dermatological effects (*e.g.*, hirsutism, redistribution of subcutaneous fat, impaired wound healing, thinning of

the skin) and suppression of the hypothalamus-pituitary-adrenal axis (see *e.g.*, Truhan, A.P. *et al.* (1989) *Annals of Allergy* 62:375-391). Many of the side effects of corticosteroid usage appear to be dose-dependent (Kimberly, R.P. (1992) *Curr. Opin. Rheumatol.* 4:325-331). Accordingly, methods and compositions that enable the use of a lower effective dosage of corticosteroids (referred to as the "steroid sparing effect") would be highly desirable to avoid unwanted side effects.

Another problem that limits the usefulness of corticosteroids is the phenomenon of steroid resistance. Certain inflammatory or immunological diseases exhibit refractoriness to steroid treatment. For example, attempts to use corticosteroid therapy to treat septic shock in humans have met with disappointing results and thus corticosteroids are not generally recommended as adjunctive therapy in severe sepsis or septic shock (see *e.g.*, Putterman, C. (1989) I*srael J. Med. Sci.* 25:332-338; Bone, R.C. and Brown, R.C. (1990) in Vincent, J.L. (ed.) "*Update in Intensive Care and Emergency Medicine 10*", Heidelberg:Springer Verlag, p. 121). Other disorders that often exhibit resistance to corticosteroid treatment include inflammatory bowel disease (see *e.g.*, Hibi, T. *et al.* (1995) *J. Gastroenterol.* 30:121-123) and graft-versus-host disease (Antin, J.H. *et al.* (1994) *Blood* 84:1342-1348; Racadot, E. *et al.* (1995) *Bone Marrow Transplantation* 15:669-677). Thus, methods and compositions that can be used to overcome or reverse corticosteroid resistance in inflammatory and immunological disorders are still needed.

Yet another disadvantage of corticosteroid therapy is the occurrence of a "steroid rebound effect" when corticosteroid administration is discontinued. A steroid rebound effect is characterized by the worsening of the inflammatory condition(s) being treated upon cessation of steroid therapy. Methods and compositions that can be used to ameliorate the steroid rebound effect are still needed.

Summary of the Invention

This invention provides methods and compositions for modulating responsiveness to corticosteroids in a subject. For example, the methods and compositions of the invention can be used to reverse steroid resistance in a subject, to thereby allow the subject to be treated with corticosteroids. The methods and

compositions of the invention also can be used to increase steroid sensitivity in a subject, to thereby achieve therapeutic effectiveness of corticosteroid treatment at lower dosages (*e.g.*, to avoid harmful side effects of high doses of corticosteroids or to allow treatment of steroid-dependent diseases with lower doses). Still further, the methods and compositions of the invention can be used to ameliorate the steroid rebound effect when a subject undergoing corticosteroid treatment is taken off corticosteroids.

In the modulatory methods of the invention, an agent which antagonizes a target that regulates production of IFN-γ in a subject is administered to the subject in combination with a corticosteroid such that responsiveness of the subject to the corticosteroid is modulated as compared to when a corticosteroid alone is administered to the subject. The target which is antagonized can be, for example, a cytokine or enzyme that regulates IFN-γ production or a cell that regulates IFN-γ production. The agent is administered at a dosage and by a route sufficient to inhibit IFN-γ production in the subject. In various embodiments, the agent and the corticosteroid are administered at the same time, the agent is administered first and then the corticosteroid is administered or the corticosteroid is administered first and then the agent is administered. The methods can be applied to prophylactic and therapeutic regimens of corticosteroid treatment.

In one embodiment, the method involves administration of an agent that is an IL-18 antagonist. The IL-18 antagonist is administered at a dosage and by a route sufficient to inhibit IL-18 activity in the subject. The IL-18 antagonist can act, for example, at the level of IL-18 synthesis, IL-18 cytokine activity or IL-18 interaction with an IL-18 receptor. In a preferred embodiment, the IL-18 antagonist is an inhibitor of a caspase family protease, preferably an Interleukin-1β Converting Enzyme (ICE) inhibitor. In another embodiment, the IL-18 antagonist is an antibody (e.g., a neutralizing antibody or a human antibody), antibody fragment (e.g., a human antibody fragment or a neutralizing antibody fragment), natural or native binding protein (e.g., human IL-18 binding protein) or engineered binding protein that binds to IL-18 or an IL-18 receptor. In another embodiment, the IL-18 antagonist may block or inhibit the formation of IL-18 by binding or interacting with pro-IL-18.

Furthermore, the invention provides a method for blocking IFN-γ production by administering both an agent which blocks caspase-1 (ICE) activation of IL-18 and at least one second agent, which blocks caspase-1 independent activation of IL-18 (e.g., a pan-caspase inhibitor, a caspase-4 inhibitor, a caspase-5 inhibitor, or an inhibitor of PR3). In another embodiment, the method involves administration of an agent that is an Interleukin-12 (IL-12) antagonist. The IL-12 antagonist is administered at a dosage and by a route sufficient to inhibit IL-12 activity in the subject. The IL-12 antagonist can act, for example, at the level of IL-12 synthesis, IL-12 cytokine activity or IL-12 interaction with an IL-12 receptor. In a preferred embodiment, the IL-12 antagonist is an antibody (e.g., J695, a neutralizing antibody or a human antibody), antibody fragment (e.g., a human antibody fragment or a neutralizing antibody fragment), natural or native binding protein (e.g., human IL-12 binding protein) or engineered binding protein that binds to IL-12 or an IL-12 receptor. In one embodiment, the IL-12 antagonist can act at the level of IL-12 synthesis by interacting or binding to an IL-12 precursor, thus preventing the formation of IL-12. In another preferred embodiment, the IL-12 antagonist is an agent that stimulates production of cyclic AMP (cAMP) in cells that produce IL-12. Examples of agent that can be used to stimulate cAMP include phosphodiesterase IV inhibitors and beta-2 agonists. In yet another embodiment, the IL-12 antagonist is a STAT4 inhibitor.

In yet another embodiment, the method involves administration of an agent that depletes or eliminates NK cells and NK-like cells (referred to herein as an "NK cell antagonist") from the subject. The NK cell antagonist is administered at a dosage and by a route sufficient to inhibit IFN-γ production in the subject. Preferred NK cell antagonists are antibodies specific for NK/NK-like cells that deplete these cells *in vivo*. Examples of preferred antibodies for use as NK cell antagonists are anti-asialo-GM1 antibodies and NK1.1 antibodies.

Another aspect of the invention pertains to a method for modulating responsiveness to corticosteroids in a subject, wherein an inhibitor of a caspase family protease, preferably ICE, is administered to the subject together with a corticosteroid, such that responsiveness of the subject to the corticosteroid is modulated as compared to when a corticosteroid alone is administered to the subject.

Yet another aspect of the invention pertains to a method for modulating responsiveness to corticosteroids in a subject, wherein an IL-12 antagonist is administered to the subject together with a corticosteroid, such that responsiveness of the subject to the corticosteroid is modulated as compared to when a corticosteroid alone is administered to the subject.

Yet another aspect of the invention pertains to a method for modulating responsiveness to corticosteroids in a subject, wherein an NK cell antagonist (e.g., an anti-NK/NK-like cell antibody) is administered to the subject together with a corticosteroid, such that responsiveness of the subject to the corticosteroid is modulated compared to when a corticosteroid alone is administered to the subject.

Still another aspect of the invention pertains to a method for modulating responsiveness to corticosteroids in a subject, wherein a subject in need of modulation of responsiveness to a corticosteroid is selected and an agent which antagonizes a target that regulates production of IFN-γ in the subject is administered to the subject such that responsiveness of the subject to a corticosteroid is modulated as compared to when a corticosteroid alone is administered to the subject. The agent is administered to the subject at a dosage and by a route sufficient to inhibit IFN-γ production in the subject. The subject that is selected can be, for example, a subject that is steroid resistant prior to treatment, a steroid-responsive subject in whom steroid sensitivity is to be increased or a subject to be taken off steroids in whom the steroid rebound effect is to be ameliorated.

The invention also provides pharmaceutical compositions for modulating responsiveness to corticosteroids in a subject. In one embodiment, a composition of the invention comprises an agent which antagonizes a target that regulates production of IFN-γ in the subject, a corticosteroid and a pharmaceutically acceptable carrier. In another embodiment, a composition of the invention comprises at least one IL-18 antagonist (such as inhibitor of a caspase family protease, preferably an ICE inhibitor, or an anti- IL-18 or anti- IL-18 receptor monoclonal antibody), a corticosteroid and a pharmaceutically acceptable carrier. In yet another embodiment, a composition of the invention comprises an IL-12 antagonist (*e.g.*, an anti-IL-12 or anti-IL-12 receptor monoclonal antibody, a phosphodiesterase IV inhibitor, a beta-2 agonist, a STAT4

inhibitor), a corticosteroid and a pharmaceutically acceptable carrier. In still another embodiment, a composition of the invention comprises an NK-cell antagonist (e.g., an anti-NK/NK-like cell antibody), a corticosteroid and a pharmaceutically acceptable carrier. The pharmaceutical compositions of the invention can be formulated for administration via a preferred route of administration for achieving a desired therapeutic effect. In one preferred embodiment, the pharmaceutical composition is formulated for topical administration. In another preferred embodiment, the pharmaceutical composition is formulated for administration by inhalation. Other preferred routes of administration include oral, intrarectal, intramuscular, subcutaneous and intravenous administration.

The invention also provides a packaged composition for use in combination therapy with corticosteroids, comprising an agent which antagonizes a target that regulates production of IFN-γ in the subject and directions instructing the administration of the composition in combination with corticosteroids. In a further embodiment, the composition also comprises a pharmaceutically effective carrier. Examples of preferred agents include ICE inhibitors, anti-IL-12 antibodies, and anti-IL-18 antibodies.

The methods and compositions of the invention can be used in the treatment of any disease or disorder in which it is desirable to modulate steroid responsiveness. In a preferred embodiments, the methods and compositions of the invention are used to treat a subject suffering from septic shock. In another embodiment, the methods and compositions of the invention are used to treat a subject suffering from Crohn's disease. In another embodiment, the methods and compositions are used to treat a subject suffering from asthma. In another embodiment, the methods and compositions are used to treat a subject suffering from an autoimmune disease or disorder. In another embodiment, the methods and compositions are used to treat a subject suffering from graft-versus-host disease or transplant rejection. In yet another embodiment, the methods and compositions are used to treat a subject suffering from an acute inflammatory disorder. In still another embodiment, the methods and compositions are used to treat a subject suffering from a chronic inflammatory disorder.

Brief Description of the Drawings

Figure 1 is a bar graph showing serum TNFα levels (in ng/ml) in wild type and ICE-deficient (ICE KO) mice treated with vehicle alone or dexamethasone (4 mg/kg) 30 minutes after LPS in the LPS/*P. acnes* septic shock model, demonstrating that the ICE-deficient mice, but not wild type mice, exhibit suppression of TNFα production and hence are steroid responsive.

Figure 2 is a bar graph showing serum TNFα levels (in ng/ml) in wild type (solid bars) and ICE-deficient (hatched bars) mice pretreated with vehicle alone or decreasing amounts of dexamethasone (0.05, 0.005 or 0.0005 mg/kg) in the LPS/*P. acnes* septic shock model, demonstrating that the ICE-deficient mice maintain steroid responsiveness to decreasing steroid dosages in contrast to the wild type mice.

Figure 3 is a bar graph showing LPS-induced serum IL-12 (in pg/ml) in B6 mice pretreated with vehicle alone or with the phosphodiesterase IV inhibitor, rolipram, demonstrating that treatment with the phosphodiesterase IV inhibitor inhibits production of IL-12.

Figure 4 is a bar graph showing serum TNFα levels (in ng/ml) in B6 mice treated with vehicle alone (saline) or an ICE inhibitor (Ac-YVAD-CHO), in combination with dexamethasone treatment, in the LPS/*P. acnes* septic shock model.

Figure 5 is a bar graph showing serum IL-6 levels (in ng/ml) in B6 mice treated with vehicle alone (saline) or an ICE inhibitor (Ac-YVAD-CHO), in combination with dexamethasone treatment, in the LPS/*P. acnes* septic shock model.

Figure 6 is a bar graph showing serum IL-1β levels (in ng/ml) in B6 mice treated with vehicle alone (saline) or an ICE inhibitor (Ac-YVAD-CHO), in combination with dexamethasone treatment, in the LPS/*P. acnes* septic shock model.

Figure 7 is a bar graph showing *E. coli* DNA induces IFNγ production in both ICE KO and wild type mice.

Figure 8a is a bar graph showing *E. coli* DNA induced IFN-γ production is inhibited by anti-IL-12 antibodies and anti-IL 18 antibodies. Figures 8b and 8c depict a line and a bar graph, respectively, of anti-mu-IL-18 antibodies blocking ec-DNA induced IFN-γ production *in vitro*. In Figure 8c, the concentration of *E.coli* DNA is 25 μg/ml and the concentration of IL-18 is 15 ng/ml.

Figure 9a is a bar graph showing *E. coli* DNA induced IFN-γ production is inhibited by a pan-caspase inhibitor, WO 1818. Figure 9b shows that exogenous IL-18 restores IFN-γ production in the presence of the pan-caspase inhibitor (WO 1818).

Figures 10a and 10b are bar graphs depicting that soluble antigen (KLH)-induced IFN-γ production by murine LNC is inhibited by anti-IL-12 and anti-IL-18 antibodies but not by a pan-caspase inhibitor, WO 1818.

Figures 11a and 11b are bar graphs showing that caspase-1 -/- mice are defective in LPS induced, but not ec-DNA-induced IFNγ production.

Figures 12a, 12b and 12c are bar graphs showing that ec-DNA-induced IFNγ production is inhibited by a pan-caspase inhibitor (z-VAD-FMK), but not by the caspase-1 inhibitor (YVAD-CHO), or the caspase-3 inhibitor (DEVD-CHO). Figure 12a shows the IFN-γ production using wild type mice. Figure 12b and 12c show the IFN-γ production using caspase-1 -/- mice.

Detailed Description of the Invention

This invention is based, at least in part, upon the discovery that ICE deficient mice, in contrast to wild type control mice, are responsive to corticosteroids after LPS challenge in a septic shock model (see Example 1). Moreover, the ICE deficient mice

show increased sensitivity to low doses of corticosteroids compared to wild type control mice, when corticosteroid treatment is given before LPS challenge in the septic shock model (see Example 2). The invention further is based, at least in part, upon the discovery that depletion of NK/NK-like cells in LPS-challenged wild type mice leads to substantially decreased IFN-γ production (compared to control untreated mice) and to substantially increased survival rates (see Example 10).

It has previously been described that administration of interferon-γ (IFN-γ) can overcome corticosteroid suppression of TNFα biosynthesis by murine macrophages (Leudke, C.E. and Cerami, A. (1990) *J. Clin. Invest.* 86:1234-1240). Moreover, ICE and other caspase family proteases can cleave the precursor form of IL-18 to its mature, active form (see Examples 4 and 14). Although not intending to be limited by mechanism, the ability to confer corticosteroid responsiveness by inhibiting ICE activity in a subject, in accordance with the present invention, is thought to result from inhibition of IL-18 processing by ICE such that production of mature IL-18 is inhibited, thereby leading to decreased production of IFN-γ in the subject. The production of IL-18 can be further reduced, for example, by also inhibiting alternate pathways of IL-18 production. These pathways include activation by caspases other than caspase-1 (e.g., caspases 4 and 5) and by the protease PR3. Moreover, IL-18 in conjunction with IL-12 stimulates NK/NK-like cells to make more IFN-γ. Thus, NK/NK-like cells are thought to form a positive feedback loop in the production of IFN-γ, which can be downmodulated by depletion or elimination of the NK/NK-like cells.

In view of the foregoing, the invention broadly provides methods and compositions for modulating responsiveness to corticosteroids in which a target that regulates production of IFN-γ is antagonized in a subject. This target that regulates production of IFN-γ, and which is antagonized, can be IL-18 (which can be antagonized, for example, indirectly by inhibiting ICE activity or directly by use of an anti-IL-18 antibody). Alternatively, another factor that regulates production of IFN-γ, such as IL-12, can be antagonized to thereby modulate corticosteroid responsiveness in the subject. Still further, an agent that depletes or eliminates NK/NK-like cells to thereby inhibit IFN-γ production can be used to modulate corticosteroid responsiveness in the subject.

So that the invention may be more readily understood, a number of terms are first defined.

As used herein, the term "corticosteroid" refers to a class of therapeutic agents useful in treatment of inflammatory conditions, including those resulting from infection, transplant rejection and autoimmune disorders. Corticosteroids include those that are naturally occurring, synthetic, or semi-synthetic in origin, and are characterized by the presence of a steroid nucleus of four fused rings, for example, as found in cholesterol, dihydroxycholesterol, stigmasterol, and lanosterol structures. Corticosteroid drugs include cortisone, cortisol, hydrocortisone (11β17-dihydroxy-21-(phosphonooxy)-pregn-4-ene3,20-dione disodium), dihydroxycortisone, dexamethasone (21-(acetyloxy)-9-fluoro-11β,17-dihydroxy-16α-methylpregna-1,4-diene-3,20-dione), and highly derivatized steroid drugs such as beconase (beclomethasone dipropionate, which is 9-chloro-11β,17,21, trihydroxy-16β-methylpregna-1, 4 diene-3, 20-dione 17,21-dipropionate). Other examples of corticosteroids include flunisolide, prednisone, prednisolone, methylprednisolone, triamcinolone, deflazacort and betamethasone.

The term "target that regulates production of IFN-γ" is intended to include chemical factors (e.g., cytokines, enzymes and the like) and cells that directly or indirectly control the synthesis of IFN-γ in a subject. Examples of factors that regulate the production of IFN-γ include IL-18 (see *e.g.*, Okamura, H. *et al.* (1995) *Nature* 378:88-91; Ushio, S. *et al.* (1996) *J. Immunol.* 156:4274-4279) and interleukin-12 (IL-12)(see *e.g.*, Schoenhaut, D. *et al.* (1992) *J. Immunol.* 148:3433; PCT Publication WO 90/05147; European Patent Application EP 433 827 A2). Examples of cells that regulate IFN-γ production include T-cells, NK and NK-like cells. The regulation of the production of IFN-γ may be either systemic or local (e.g., at the site of inflammation).

As used herein, agents that "antagonize" a factor are intended to include agents that inhibit the activity of the factor and agents that downregulate (*i.e.*, inhibit) the synthesis or production of the factor. The term "IL-18" refers to a cytokine having an amino acid sequence as disclosed in Okamura, H. *et al.* (1995) *Nature* 378:88-91 (mouse) or Ushio, S. *et al.* (1996) *J. Immunol.* 156:4274-4279 (human), and other

mammalian homologues thereof. The cytokine IL-18 has also been referred to in the art as Interferon γ Inducing Factor (IGIF) and IL-1γ.

The term "IL-18 antagonist" is intended to include agents that inhibit the synthesis or production of IL-18, agents that inhibit the activity of IL-18 once synthesized, agents that inhibit the interaction of IL-18 with an IL-18 receptor and agents that inhibit the activity of an IL-18 receptor. Examples of IL-18 antagonists include inhibitors of caspase family proteases (*e.g.*, ICE inhibitors, pan-caspase inhibitors, inhibitors of caspase-4, and inhibitors of caspase-5), inhibitors of other IL-18 activating enzymes (e.g., inhibitors of the protease, PR3), antibodies (e.g., chimeric antibodies, humanized antibodies, human antibodies), antibody fragments (e.g., chimeric antibody fragments, humanized antibody fragments, or human antibody fragments), native or natural binding proteins (e.g., human Il-18 binding protein) and engineered binding proteins that bind to either IL-18 or an IL-18 receptor. Advantageously, the antibodies and antibody fragments of the invention are neutralizing. IL-18 antagonists also include agents which inhibit the production of IL-18 by binding or interacting with pro-IL-18.

The term "interleukin-12 (IL-12)" refers to a cytokine having an amino acid sequence as disclosed in Schoenhaut, D. *et al.* (1992) *J. Immunol.* 148:3433, PCT Publication WO 90/05147; and European Patent Application EP 433 827 A2, and other mammalian homologues thereof.

The term "IL-12 antagonist" is intended to include agents that inhibit the synthesis or production of IL-12, agents that inhibit the activity of IL-12 once synthesized, agents that inhibit the interaction of IL-12 with an IL-12 receptor and agents that inhibit the activity of an IL-12 receptor. Examples of IL-12 antagonists include antibodies (e.g., J695, chimeric antibodies, humaized antibodies and human antibodies), antibody fragments (e.g., chimeric antibody fragments, humanized antibody fragments, and human antibody fragments), native or natural binding proteins, and engineered binding proteins that bind to either IL-12 or an IL-12 receptor, agents that stimulate intracellular production of cAMP in cells that produce IL-12 (such as phosphodiesterase IV inhibitors or beta-2 agonists) and agents that inhibit STAT4. Advantageously, the antibodies and antibody fragments of the invention are neutralizing.

The term "caspase family protease" is intended to include members of the caspase proteases as described in Alnemri, E. *et al.* (1996) *Cell* 87:171, including caspase-1 (ICE), caspase-2 (ICH-1), caspase-3 (CPP32, Yama, apopain), caspase-4 (TX, ICH-2, ICE_{rel}-II), caspase-5 (ICE_{rel}-III, TY), caspase-6 (Mch2), caspase-7 (Mch3, ICE-LAP3, CMH-1), caspase-8 (MACH, FLICE, Mch5), caspase-9 (ICE-LAP6, Mch6) and caspase-10 (Mch4). Furthermore, a "caspase family protease" is intended to include any protein that shares greater than 20% amino acid sequence identity with ICE in the active domains of the protease (*i.e.*, active domains of the p10 and p20 subunits of ICE), contains the peptide sequence glutamine-alanine-cysteine-X-glycine (QACXG), wherein the cysteine (C) is the catalytically active cysteine residue and X denotes any amino acid, and contains the sequence serine-histidine-glycine (SHG), located N-terminal to the QACXG motif, in which the histidine (H) is the catalytically essential histidine residue. Caspase family proteases typically demonstrate a strong preference for hydrolysis of peptide bonds immediately following an acidic amino acid (*i.e.*, aspartic acid or glutamic acid). The term includes proteases which are inhibited by pan-caspase inhibitors, whose structures and/or sequences may not yet be elucidated. An example of a pan caspase inhibitor is z-VAD-FMK, (Miwa et al. (1998) *Nature Medicine* 4:1287-1292).

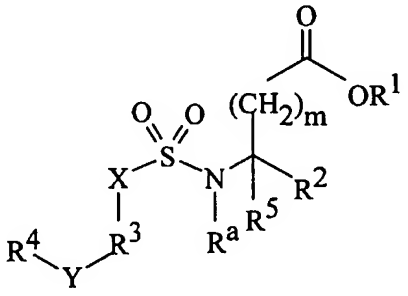
Caspase family proteases are known in humans and other organisms including mice and *Caenorhabditis elegans*. Examples of caspase family proteases include, for example, Ich-1 (Wang, L. *et al.* (1994) *Cell* 78:739-750); ICH-2 (Kamens, J. *et al.* (1995) *J. Biol. Chem.* 270:15250-15256); Mch2 (Fernandes-Alnemri, T. *et al.* (1995) *Cancer Res.* 55:2737-2742); CPP32 (Fernandes-Alnemri, T. *et al.* (1994) *J. Biol. Chem.* 269:30761-30764); Yama/CPP32β (Tewari, M. *et al.* (1995) *Cell* 81:801-809); the product of the mouse gene *Nedd2* (Kumar, S. *et al.* (1992) *Biochem. Biophys. Res. Commun.* 185:1155-1161; Kumar, S. *et al.*. (1994) *Genes Dev.* 8:1613-1626); the product of the *C. elegans* gene, *ced-3* (Yuan, J. *et al.* (1993) *Cell* 75:641-652); the human protein TX (Faucheu, C., *et al.*, (1995) *EMBO J.* 14:1914-1922); ICE_{rel}II and ICE $_{rel}$III (Munday, N.A. *et al.* (1995) *J. Biol. Chem.* 270:15870-15876).

The term "interleukin-1β converting enzyme (ICE)" is intended to refer to a protease having an amino acid sequence as disclosed in Cerretti, D.P. *et al.* (1992) *Science* 256:97-100 (human) or Nett, M.A. *et al.* (1992) *J. Immunol.* 149:3254-3259 (mouse), and other mammalian homologues thereof. The term "ICE" includes caspase-1.

The term "ICE inhibitor" is intended to include chemical agents that inhibit the proteolytic activity of ICE. Examples of ICE inhibitors are known in the art, including, for example, agents disclosed in U.S. Patent No. 5,585,357 (pyrazolyl derivatives); U.S. Patent No. 5,677,283 (pyrazolyl derivatives); U.S. Patent No. 5,656,627 (inhibitors comprising a hydrogen bonding group, a hydrophobic group and an electronegative group); U.S. Patent No. 5,411,985 (gamma-pyrone-3-acetic acid compounds); U.S. Patent No. 5,430,128 (tripeptidyl derivatives); U.S. Patent No. 5,434,248 (tripeptidyl compounds); U.S. Patent No. 5,565,430 (N,N'-diacylhydrazinoacetic acid compounds); U.S. Patent No. 5,416,013 (peptidyl derivatives); PCT Publication WO 94/21673 (alpha-ketoamide derivatives); PCT Publication WO 97/22619 (N-acylamino compounds); PCT Publication WO 97/22618 (amino acid or di- or tripeptide amide derivatives); PCT Publication WO 95/35308 (inhibitors comprising a hydrogen bonding group, a hydrophobic group and an electronegative group); PCT Publication WO 93/14777 (peptidyl derivatives); PCT Publication WO 93/16710 (peptidyl derivatives); PCT Publication WO 95/05152 (substituted ketone derivatives); PCT Publication WO 94/03480 (peptidyl 4-amino-2, 2-difluoro-3-oxo-1, 6-hexanedioic acid derivatives); PCT Publication WO 94/00154 (peptidyl derivatives); PCT Publication WO 93/05071 (peptidyl derivatives); European Application EP 519 748 (peptidyl derivatives); European Application EP 590 650 (cyclopropene derivatives); European Application EP 628 550 (pyridazines); European Application EP 644 198 (alpha-heteroaryloxymethyl ketones); European Application EP 644 197 (peptidic phosphinyloxymethyl ketones); European Patent Application EP 547 699 (peptidyl derivatives); Great Britain Application GB 2,278,276 (gamma-pyrone-3-acetic compounds); and Canadian Application 2,109,646 (para-nitroanilide peptides). Succinamide ICE inhibitors, disclosed in U.S. Provisional Application Serial No. 60/084,320, are also included. The

present invention encompasses use of the ICE inhibitors disclosed in any of the foregoing publications in the methods described herein.

Additional preferred ICE inhibitors for use in the methods of the invention include sulfonamide substituted aspartic acid ICE inhibitors having the formula I:



I

wherein

R^1 is hydrogen, C_1-C_6alkyl, or benzyl;

R^2 is -CHO, -COR^a, or -CN;

each R^a is independently hydrogen or C_1-C_6alkyl;

X is a bond, CH_2, CHR^5, NH, NR^5, or O;

R^3 is aryl, substituted-aryl, heteroaryl, substituted-heteroaryl, cycloalkyl, substituted-cycloalkyl, heterocycle, or substituted-heterocycle;

Y is absent, NR^5, CO, S, O, SO_2, -$O(CHR^5)_n$-, CHR^5, NR^5CO, NCR^5, $CONR^5$, $OCHR^5$, CHR^5O, $SCHR^5$, CHR^5S, SO_2NR^5, C_1-C_6alkyl, NR^5SO_2, CH_2CHR^5, CHR^5CH_2, $COCH_2$, or CH_2CO;

R^4 is absent, aryl, substituted-aryl, C_1-C_8alkyl, heteroaryl , substituted-heteroaryl, cycloalkyl, C_1-C_6alkyl, substituted-cycloalkyl, heterocycloalkyl, or substituted-heterocycloalkyl;

each R^5 is independently hydrogen, C_1-C_6alkyl, aryl, -$(CH_2)_n$aryl, or -$(CH_2)_n$cycloalkyl;

each n is independently 0 to 5, m is 1 or 2, and the pharmaceutically acceptable salts, esters, amides, and prodrugs thereof.

In one embodiment of the invention, R^2 is CHO.

In another embodiment of the invention, R^1 is hydrogen.

In another embodiment of the invention, R^a is hydrogen.

In another embodiment of the invention, X is a bond.

In another embodiment of the invention, R^3 is phenyl or substituted phenyl.

In another embodiment of the invention, Y is a bond.

In another embodiment of the invention, Y is O.

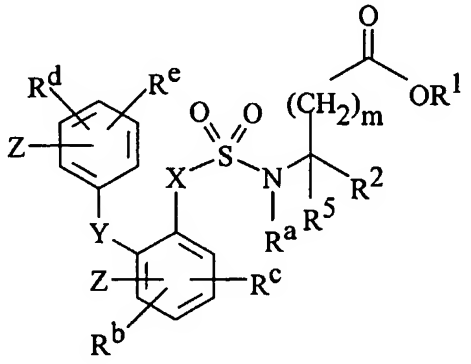
In another embodiment of the invention, Y is CH_2.

In another embodiment of the invention, R^4 is phenyl or substituted phenyl.

In another embodiment of the invention, R^2 is CHO, R^a is H, R^1 is hydrogen, X is a bond, R^3 and R^4 are phenyl or substituted phenyl, and Y is a bond, CH_2, or O.

In another embodiment of the invention, m is 1 and R^5 is hydrogen.

Other preferred sulfonamide substituted ICE inhibitors have the Formula II



II

wherein

R^1 is hydrogen, C_1-C_6alkyl, or benzyl;

R^2 is -CHO, -COR^a, or -CN;

each R^a is independently hydrogen or C_1-C_6alkyl;

X is a bond, CH_2, CHR^5, NH, NR^5, or O;

Y is a bond, NR^5, CO, S, O, SO_2, CHR^5, NR^5CO, $CONR^5$, $OCHR^5$, CHR^5O, -$O(CHR^5)_n$-, $SCHR^5$, CHR^5S, SO_2NR^5, NR^5SO_2, CH_2CHR^5, CHR^5CH_2, $COCH_2$, or CH_2CO;

each R^5 is independently hydrogen, C_1-C_6alkyl, aryl, or -$(CH_2)_n$aryl;

each n is independently 0 to 5;

m is 1 or 2;

Each Z is independently hydrogen, or an aryl, substituted aryl, heteroaryl, substituted heteroaryl, cycloalkyl, substituted cycloalkyl, heterocycle, or substituted

heterocycle group that is fused to the phenyl group that contains Z as a substituent;

R^b, R^c, R^d, and R^e are each independently hydrogen, C_1-C_6alkyl, C_1-C_6alkoxy, -OH, C_1-C_6 thioalkoxy, halogen, trifluoromethyl, dialkylamino, $-NO_2$, -CN, $-CF_3$, $-CO_2$alkyl, $-SO_3H$, -CHO, -COalkyl, -CONH-alkyl, -CONHR9, $-CON(alkyl)_2$, $-(CH_2)_n-NH_2$, $-(CH_2)_n$-NH-alkyl, -NHR9, $-NHCOR^9$, $-(CH_2)_nOH$, $-(CH_2)_nCONH_2$, or $-(CH_2)_nCO_2H$; and

R9 is hydrogen or C_1-C_6alkyl, and the pharmaceutically acceptable salts, esters, amides, and prodrugs thereof.

In another embodiment with respect to the compounds of Formula II, R^1 is hydrogen.

In another embodiment with respect to the compounds of Formula II, R^2 is CHO.

In another embodiment with respect to the compounds of Formula II, R^a is hydrogen.

In another embodiment with respect to the compounds of Formula II, X is a bond.

In another embodiment with respect to the compounds of Formula II, Y is a bond, O, or CH_2.

In another embodiment with respect to the compounds of Formula II, R^b and R^c are hydrogen.

In another embodiment with respect to the compounds of Formula II, wherein R^b, R^c, and R^d are hydrogen and R^e is C_1-C_6 alkyl.

In another preferred embodiment with respect to the compounds of Formula II, R^b or R^c is located at the para position of the phenyl ring with respect to X and R^b or R^c is $-OCH_3$.

In another embodiment with respect to the compounds of Formula II, m is 1 and R^5 is hydrogen.

Preferred compounds include:

3-(Biphenyl-2-sulfoamino)-4-oxo-butyric acid;

3-(2-Benzyl-benzenesulfonylamino)-4-oxo-butyric acid;

4-Oxo-3-(2-phenoxy-benzenesulfonylamino)-butyric acid;

4-Oxo-3-(2-p-tolyloxy-benzenesulfonylamino)-butyric acid;

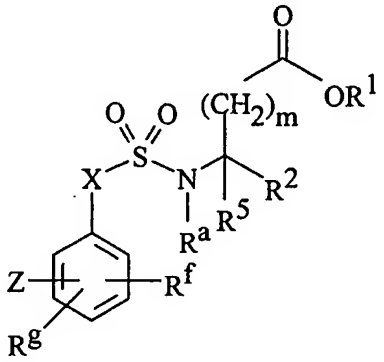
3-[2-(4-Isopropyl-phenoxy)-benzenesulfonylamino]-4-oxo-butyric acid;

4-Oxo-3-(2-m-tolyloxy-benzenesulfonylamino)-butyric acid;

3-[2-(3-Isopropyl-phenoxy)-benzenesulfonylamino]-4-oxo-butyric acid; and

3-(4'-Methyl-biphenyl-2-sulfonylamino)-4-oxo-butyric acid.

Other ICE inhibitors include compounds of the Formula III



III

wherein

R^1 is hydrogen, C_1-C_6alkyl, or benzyl;

R^2 is -CHO, -COR^a, or -CN;

each R^a is independently hydrogen or C_1-C_6alkyl;

X is a bond, CH_2, CHR^5, NH, NR^5, or O;

R^5 is hydrogen, C_1-C_6alkyl, aryl, or -$(CH_2)_n$aryl;

each n is independently 0 to 5;

m is 1 or 2;

Z is absent, or an aryl, substituted aryl, heteroaryl, substituted heteroaryl, cycloalkyl, substituted cycloalkyl, heterocycle, or substituted heterocycle group that is fused to the phenyl group that contains Z as a substituent;

R^f, R^g, are each independently hydrogen, C_1-C_6alkyl, hydroxy, halogen, trifluoromethyl, dialkylamino, -NO_2, -CN, -CO_2H, -CO_2alkyl, -SO_3H, -CHO, -COalkyl, -$CONH_2$, -$CONH(CH_2)_n$aryl, -$CONH(CH_2)_n$-substituted-aryl, -CONH-alkyl, -CONHR^q, -CON(alkyl)$_2$, -$(CH_2)_n$-NH_2, -$(CH_2)_n$-NH-alkyl, -NHR^q, -NHCOR^q, -OR^q, -SR^q, or -$(CH_2)_n$aryl; and

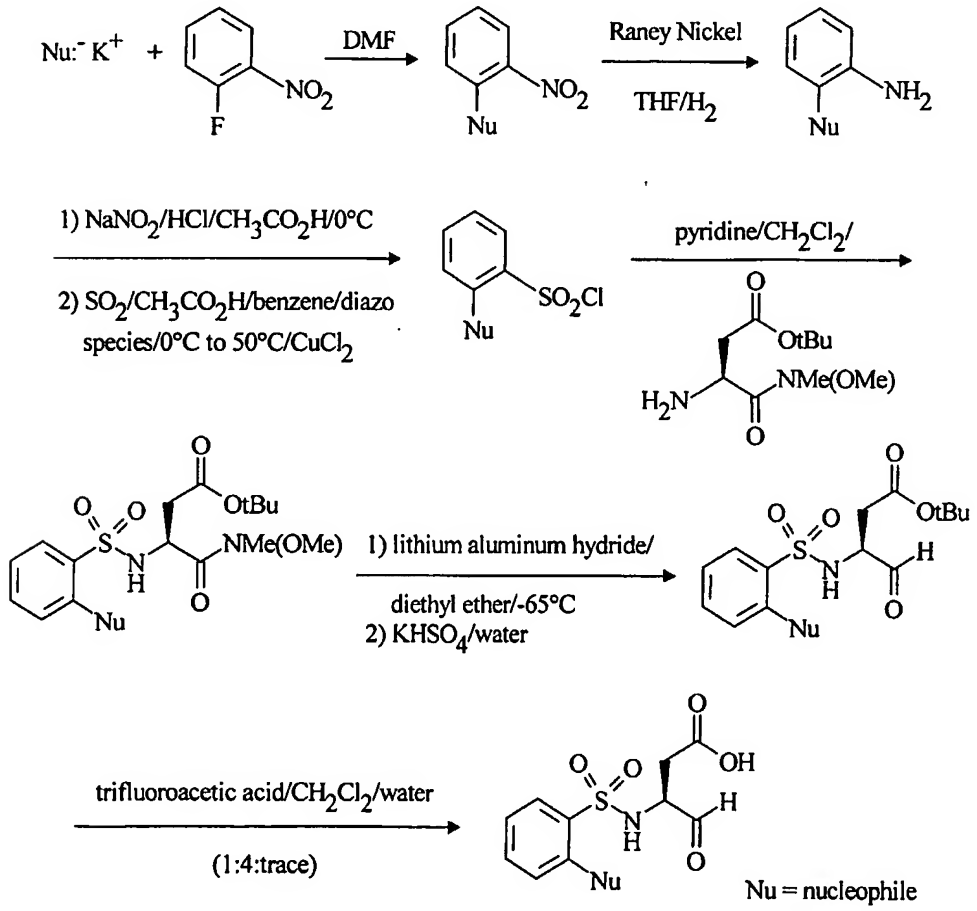
R^q is hydrogen or C_1-C_8alkyl, and the pharmaceutically acceptable salts, esters, amides, and prodrugs thereof.

In a preferred embodiment of the compounds of Formula III, R^f is ortho to X on the phenyl ring, and R^g is hydrogen.

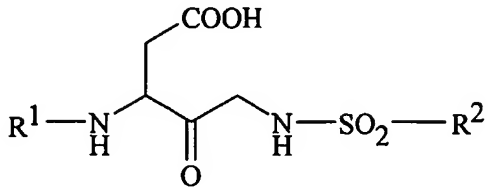
In a preferred embodiment of the compounds of Formula III, Z is hydrogen, m is 1, R^5 is hydrogen, and R^a is hydrogen.

In a preferred embodiment of the compounds of Formula III, the compound is 3-benzenesulfonylamino-4-oxo-butyric acid.

The above-described sulfonamide substituted aspartic acid ICE inhibitors can be made generally as follows:

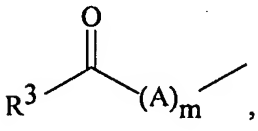


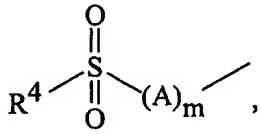
Other sulfonamide ICE inhibitors that can be used in the invention are compounds of Formula IV:

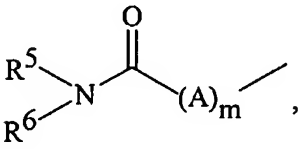


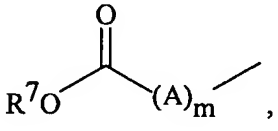
IV

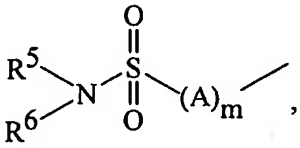
wherein R^1 is

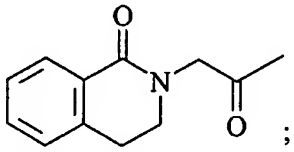












;

R^3 is hydrogen,

C_1-C_6 alkyl,

-$(CH_2)_n$ aryl, or

-$(CH_2)_n$ heteroaryl;

R^4 is C_1-C_6 alkyl,

-$(CH_2)_n$ aryl, or

-$(CH_2)_n$ heteroaryl;

R^5 and R^6 are each independently hydrogen,

C_1-C_6 alkyl,

-$(CH_2)_n$ aryl, or

-$(CH_2)_n$ heteroaryl;

R^7 is C_1-C_6 alkyl,

-$(CH_2)_n$ aryl, or

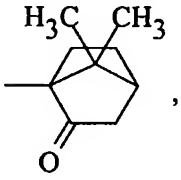
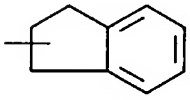
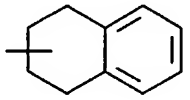
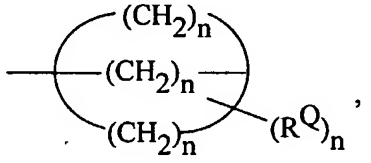
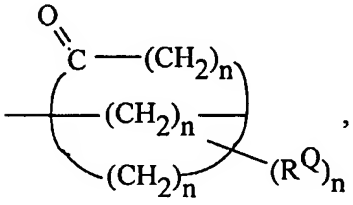
$-(CH_2)_n$ heteroaryl;

each n is 0 to 6;

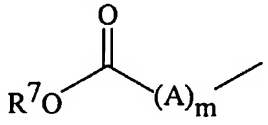
each m is 0, 1, 2, or 3;

A is alanine, leucine, isoleucine, proline, phenylalanine, glycine, tyrosine, serine, threonine, tryptophan, cysteine, methionine, valine, asparagine, glutamine, aspartic acid, lysine, glutamic acid, arginine, or histidine;

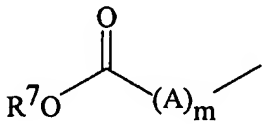
R^2 is $-(CH_2)_n$-Z; and

Z is aryl, heteroaryl, cycloalkyl, C_1-C_6alkyl,  ,  ,  ,  ,  , fluorenyl, substituted fluorenyl, substituted aryl, substituted heteroaryl, or substituted cycloalkyl, and the pharmaceutically acceptable salts, esters, amides, and prodrugs thereof.

In a preferred embodiment of the compounds of Formula IV, R^1 is

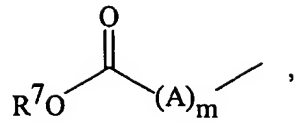
 .

In another preferred embodiment of the compounds of Formula IV, R^1 is

 ,

m is 0, and R^7 is $-(CH_2)_n$ aryl.

In another preferred embodiment of the compounds of Formula IV, R^1 is



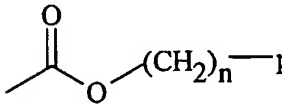
m is 0, and R^7 is -CH_2 aryl.

In another preferred embodiment of the compounds of Formula IV, R^2 is -$(CH_2)_n$ aryl.

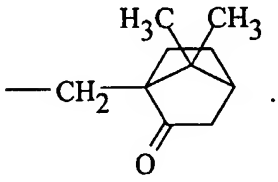
In another preferred embodiment of the compounds of Formula IV, aryl is phenyl or naphthyl.

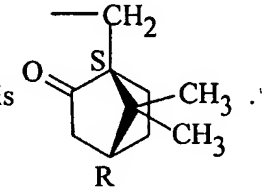
In another preferred embodiment of the compounds of Formula IV, R^2 is -$(CH_2)_n$-cycloalkyl.

In another preferred embodiment of the compounds of Formula IV, R^1 is

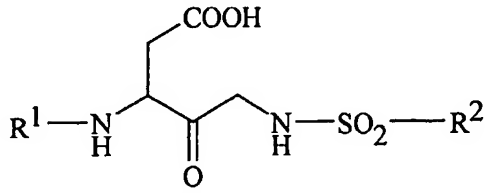
 phenyl or -SO_2-phenyl.

In another preferred embodiment of the compounds of Formula IV, R^2 is



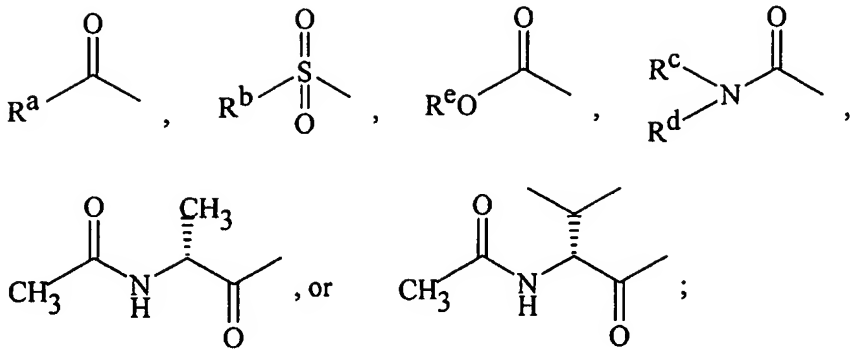
In another preferred embodiment of Formula IV, R^2 is  .

Other sulfonamide ICE inhibitors include compounds of the Formula V

 V

wherein R^2 is -CH_2CH_2- aryl, -CH_2- cycloalkyl, -CH_2CH_2- cycloalkyl, or -CH_2CH_2- heteroaryl;

R^1 is



R^a is $-(CH_2)_n$- aryl or $-(CH_2)_n$ heteroaryl;

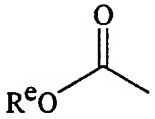
R^b is aryl or heteroaryl;

R^c is $-CH_2$ aryl or aryl;

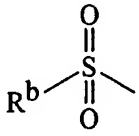
R^d is hydrogen or C_1-C_6 alkyl;

R^e is $-CH_2$ aryl or $-CH_2$ heteroaryl; and the pharmaceutically acceptable salts, esters, amides, and prodrugs thereof.

In a preferred embodiment of the compounds of Formula V R^1 is



In another preferred embodiment of the compounds of Formula V, R^1 is



In another preferred embodiment of the compounds of Formula V, R^e is $-(CH_2)_n$ aryl.

In another preferred embodiment of the compounds of Formula V, aryl is phenyl or naphthyl.

In another preferred embodiment of the compounds of Formula V, R^b is aryl.

Preferred compounds include:

3-Benzyloxycarbonylamino-4-oxo-5-(2-phenoxy-ethanesulfonylamino)-pentanoic acid;

3-Benzyloxycarbonylamino-4-oxo-5-(3-phenyl-propane-1-sulfonylamino)-pentanoic acid;

3-Benzenesulfonylamino-4-oxo-5-(2-phenylethane-1-sulfonylamino)-pentanoic acid;

5-Benzenesulfonylamino-3-benzyloxycarbonylamino-4-oxo-pentanoic acid;

3-Benzyloxycarbonylamino-5-methanesulfonylamino-4-oxo-pentanoic acid;

3-Benzyloxycarbonylamino-5-(naphthalene-1-sulfonylamino)-4-oxo-pentanoic acid;

3-Benzyloxycarbonylamino-5-(2-cyclohexyl-ethanesulfonylamino)-4-oxo-pentanoic acid;

3-Benzyloxycarbonylamino-5-(2-naphthalen-1-yl-ethanesulfonylamino)-4-oxo-pentanoic acid;

3-Benzyloxycarbonylamino-5-(7,7-dimethyl-2-oxo-bicyclo[2.2.1]hept-1-(R)-ylmethanesulfonylamino)-4-oxo-pentanoic acid;

3-Benzyloxycarbonylamino-5-(indan-1-ylmethanesulfonylamino)-4-oxo-pentanoic acid;

3-Benzyloxycarbonylamino-5-(9-fluoro-9H-fluoren-9-ylmethanesulfonylamino)-4-oxo-pentanoic acid;

3-Benzyloxycarbonylamino-5-(7,7-dimethyl-2-oxo-bicyclo[2.2.1]hept-1-(S)-ylmethanesulfonylamino)-4-oxo-pentanoic acid;

3-[2-(2-Benzyloxycarbonylamino-3-methyl-butyrylamino)-propionylamino]-4-oxo-5-(2-phenyl-ethanesulfonylamino)-pentanoic acid;

3-[2-(2-Benzyloxycarbonylamino-4-carboxy-butyrylamino)-3-methyl-butyrylamino]-4-oxo-5-(2-phenyl-ethanesulfonylamino)-pentanoic acid;

3-{2-[4-Carboxy-2-(3-phenyl-propionylamino)-butyrylamino]-3-methyl-butyrylamino}-4-oxo-5-(2-phenyl-ethanesulfonylamino)-pentanoic acid;

3-(2-{2-[2-Acetylamino-3-(4-hydroxy-phenyl)-propionylamino]-4-carboxy-butyrylamino}-3-methyl-butyrylamino)-4-oxo-5-(2-phenyl-ethanesulfonylamino)-pentanoic acid;

3-(2-Acetylamino-3-methyl-butyrylamino)-5-(7,7-dimethyl-2-oxo-bicyclo[2.2.1]hept-1-(S)-ylmethanesulfonylamino)-4-oxo-pentanoic acid;

3-(2-Acetylamino-propylamino)-5-(7,7-dimethyl-2-oxo-bicyclo[2.2.1]hept-1-(S)-ylmethanesulfonylamino)-4-oxo-pentanoic acid;

3-[2-(2-Benzyloxycarbonylamino-3-methyl-butyrylamino)-propionylamino]-5-(7,7-dimethyl-2-oxo-bicyclo[2.2.1]hept-1-ylmethanesulfonylamino)-4-oxo-pentanoic acid;

3-{2-[4-Carboxy-2-(3-phenyl-propionylamino)-butyrylamino]-3-methyl-butyrylamino}-5-(7,7-dimethyl-2-oxo-bicyclo[2.2.1]hept-1-ylmethanesulfonylamino)-4-oxo-pentanoic acid;

3-(2-{2-[2-Acetylamino-3-(4-hydroxy-phenyl)-propionylamino]-4-carboxy-butyrylamino}-3-methyl-butyrylamino)-5-(7,7-dimethyl-2-oxo-bicyclo[2.2.1]hept-1-ylmethanesulfonylamino)-4-oxo-pentanoic acid;

3-[2-(2-Benzyloxycarbonylamino-4-carboxy-butyrylamino)-3-methyl-butyrylamino]-5-(7,7-dimethyl-2-oxo-bicyclo[2.2.1]hept-1-ylmethanesulfonylamino)-4-oxo-pentanoic acid;

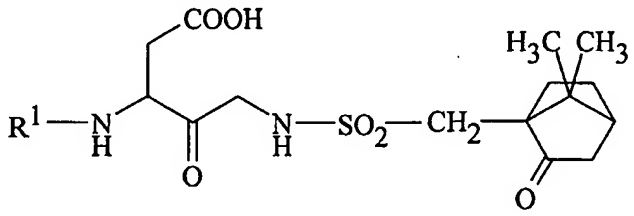
3-(1,2,3,4-tetrahydro-1-oxo-isoquinoline-2-yl)-acetanino-5-benzenesulfonylamino-4-hydroxy-pentanoic acid;

(S)-5-(Bicyclo[2.2.1]hept-1-ylmethanesulfonylamino)-4-oxo-3-[2-(1-oxo-3,4-dihydro-1H-isoquinolin-2-yl)-acetylamino]-pentanoic acid;

(S)- 4-Oxo-3-[2-(1-oxo-3,4-dihydro-1H-isoquinolin-2-yl)-acetylamino]-5-(2-phenyl-ethanesulfonylamino)-pentanoic acid; and

4-Oxo-3-[2-(1-oxo-3,4-dihydro-1H-isoquinolin-2-yl)-acetylamino]-5-phenylmethanesulfonylamino-pentanoic acid.

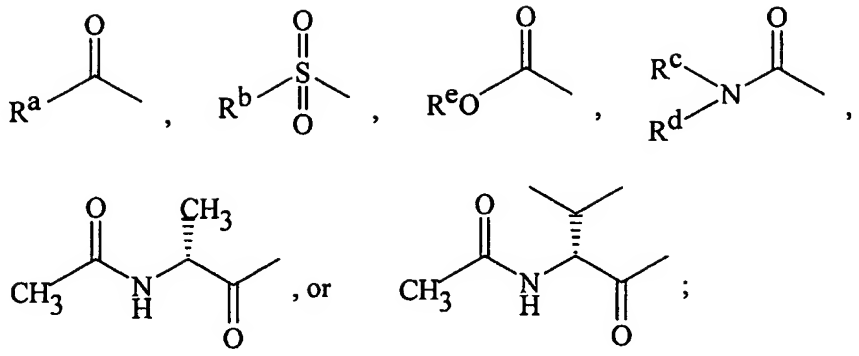
Other sulfonamide ICE inhibitors include compounds of the Formula VI:



VI

wherein

R^1 is



R^a is $-(CH_2)_n$- aryl or $-(CH_2)_n$ heteroaryl;

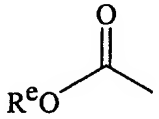
R^b is aryl or heteroaryl;

R^c is $-CH_2$ aryl or aryl;

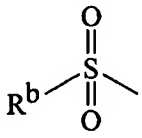
R^d is hydrogen or C_1-C_6 alkyl;

R^e is $-CH_2$ aryl or $-CH_2$ heteroaryl; and the pharmaceutically acceptable salts, esters, amides, and prodrugs thereof.

In a preferred embodiment of the compounds of Formula VI, R^1 is



In a preferred embodiment of the compounds of Formula VI, R^1 is



In another preferred embodiment of the compounds of Formula VI, R^e is $-(CH_2)_n$ aryl.

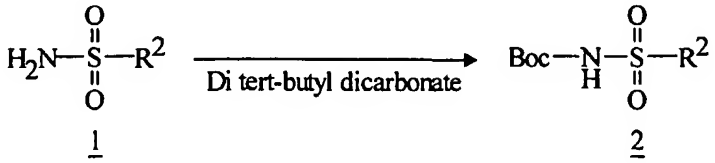
In another preferred embodiment of the compounds of Formula VI, aryl is phenyl or naphthyl.

In another preferred embodiment of the compounds of Formula VI, R^b is aryl.

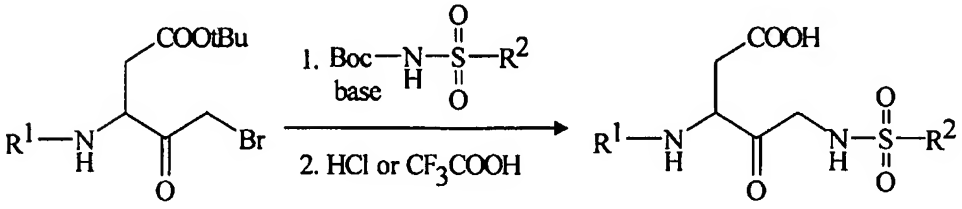
The above-described compounds of Formulas IV, V or VI can be prepared generally by converting the appropriate starting sulfonamide 1 to Boc sulfonamide 2 using a reagent such as di-tert-butyl dicarbonate. Boc sulfonamide 2 may then be reacted with the

appropriately substituted aspartic acid bromomethylketone β tert-butyl ester 3 in the presence of a base, followed by treatment with acid to give the desired product 4.

Scheme 1



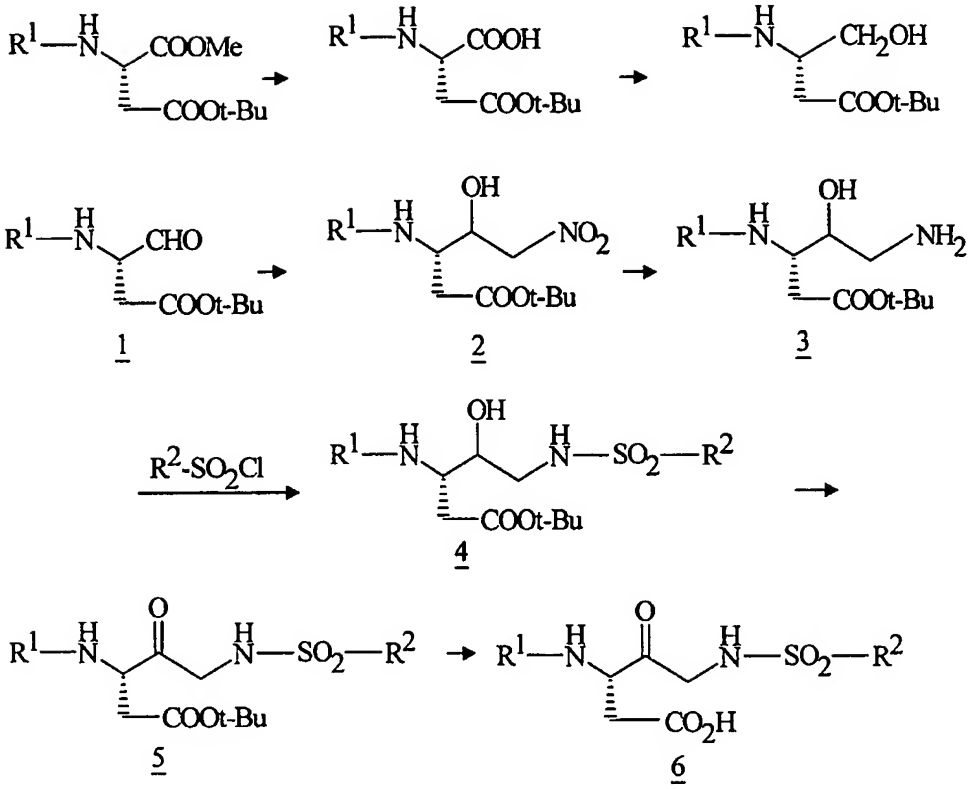
1 2



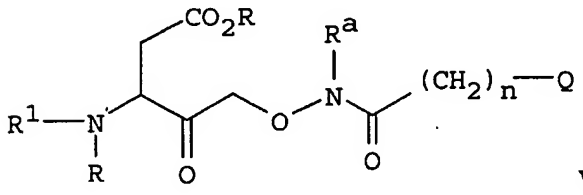
3 4

Alternatively, compounds of Formulas IV, V or VI can be prepared generally by reaction of the appropriately substituted aspartic acid aldehyde 1 with nitromethane in the presence of a base such as potassium tert-butoxide to give nitro alcohol 2. Reduction of 2 to the amine 3, followed by reaction with the appropriate sulfonyl chloride gives 4 which may be oxidized to the ketone 5 with a reagent such as Dess Martin periodinane or by a Swern oxidation. Acidic deprotection of the t-butyl ester with HCl or trifluoroacetic acid gives the desired product 6.

Scheme 2

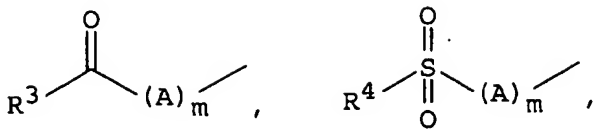


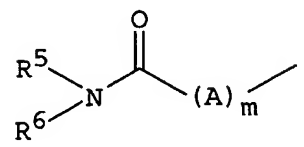
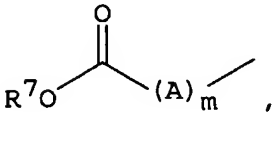
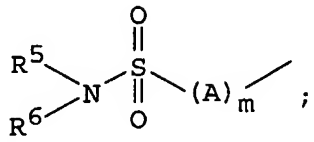
Still other ICE inhibitor compounds that can be used in the invention include hydroxamate compounds, including compounds of the formula VII:



VII

wherein R^1 is





or

;

each R is independently hydrogen or C_1-C_6alkyl;

R^3 is hydrogen, C_1-C_6alkyl, -$(CH_2)_n$aryl, -$(CH_2)_n$heteroaryl, -$(CH_2)_p$-X-aryl, or -$(CH_2)_p$-X-heteroaryl;

R^4 is C_1-C_6alkyl, -$(CH_2)_n$aryl, -$(CH_2)_n$heteroaryl, -$(CH_2)_j$-X-aryl, or -$(CH_2)_j$-X-heteroaryl;

R^5 and R^6 are each independently hydrogen, C_1-C_6alkyl, -$(CH_2)_n$aryl, -$(CH_2)_n$heteroaryl, -$(CH_2)_j$-X-aryl, or -$(CH_2)_j$-X-heteroaryl;

R^7 is C_1-C_6alkyl, -$(CH_2)_p$aryl, -$(CH_2)_p$heteroaryl, -$(CH_2)_j$-X-aryl, or -$(CH_2)_j$-X-heteroaryl;

each n is independently 0 to 6;

each p is independently 1 to 6;

each j is independently 2 to 6;

each m is 0 to 2;

A is alanine, valine, serine, threonine, glutamic acid, lysine, arginine, histidine, glutamine, or alpha amino butyric acid;

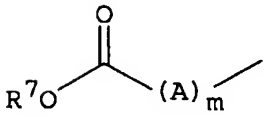
R^a is hydrogen, C_1-C_6alkyl, or -$(CH_2)_n$phenyl;

X is O or S; and

Q is C_1-C_6alkyl, -$(CH_2)_n$aryl, -$(CH_2)_n$heteroaryl, and the pharmaceutically acceptable salts, esters, amides, and prodrugs thereof.

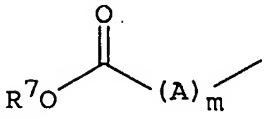
In one embodiment of the compounds of Formula VII, each R is hydrogen.

In another embodiment of the compounds of Formula VII, R^1 is



and m is 0.

In another embodiment of the compounds of Formula VII, R^1 is



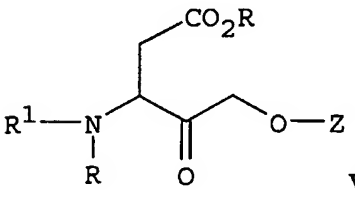
m is 0, and R^7 is $-(CH_2)_n$aryl.

In another embodiment of the compounds of Formula VII, Q is $-(CH_2)_n$phenyl or $-(CH_2)_n$naphthyl.

In another embodiment of the compounds of Formula VII, R^a is hydrogen or methyl.

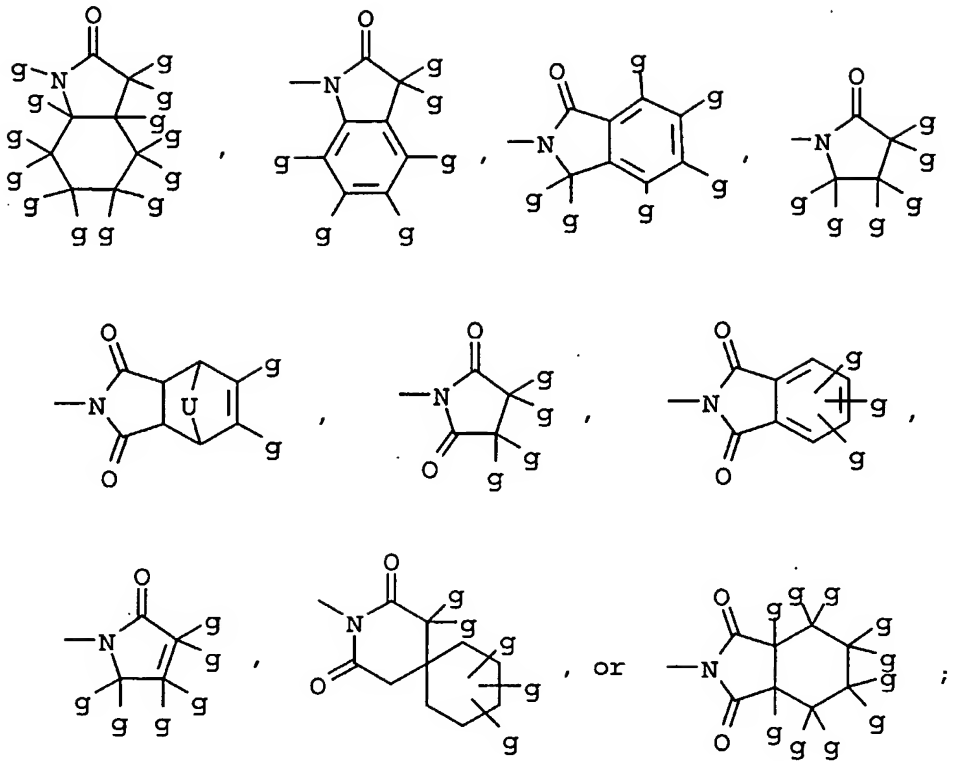
In another embodiment of the compounds of Formula VII, Q is $-CH_2$-phenyl, $-CH_2$-naphthyl, $-CH_2CH_2$-phenyl, or $-CH_2CH_2$-naphthyl.

Other hydroxamate ICE inhibitor compounds include compounds having the Formula VIII



VIII

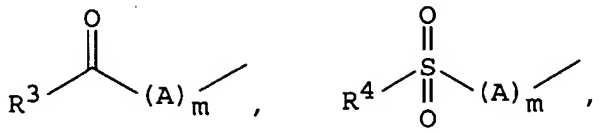
wherein Z is

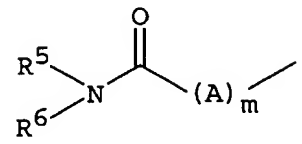
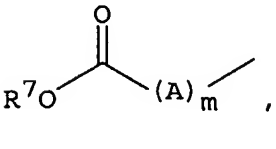
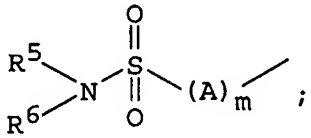


each g is independently hydrogen, C_1-C_6alkyl, C_1-C_6alkoxy, -$(CH_2)_nCO_2R$, -$(CH_2)_n$aryl, -aryl, -$(CH_2)_n$heteroaryl, or -heteroaryl;

U is O or CH_2;

R^1 is





or

;

each R is independently hydrogen or C_1-C_6alkyl;

R^3 is hydrogen, C_1-C_6alkyl, -$(CH_2)_n$aryl, -$(CH_2)_n$heteroaryl, -$(CH_2)_p$-X-aryl, or -$(CH_2)_p$-X-heteroaryl;

R^4 is C_1-C_6alkyl, -$(CH_2)_n$aryl, -$(CH_2)_n$heteroaryl, -$(CH_2)_j$-X-aryl, or -$(CH_2)_j$-X-heteroaryl;

R^5 and R^6 are each independently hydrogen, C_1-C_6alkyl, -$(CH_2)_n$aryl, -$(CH_2)_n$heteroaryl, -$(CH_2)_j$-X-aryl, or -$(CH_2)_j$-X-heteroaryl;

R^7 is C_1-C_6alkyl, -$(CH_2)_p$aryl, -$(CH_2)_p$heteroaryl, -$(CH_2)_j$-X-aryl, or -$(CH_2)_j$-X-heteroaryl;

each n is independently 0 to 6;

each p is independently 1 to 6;

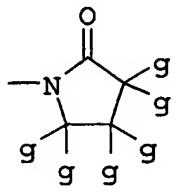
each j is independently 2 to 6;

each m is 0 to 2;

A is alanine, valine, serine, threonine, glutamic acid, lysine, arginine, histidine, glutamine, or alpha amino butyric acid; and

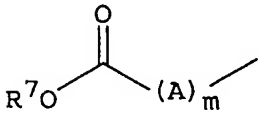
X is O or S, and the pharmaceutically acceptable salts, esters, amides, and prodrugs thereof.

In one embodiment of the compounds of Formula VIII, Z is



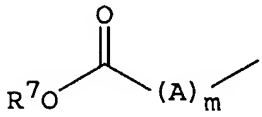
and each g is hydrogen.

In another embodiment of the compounds of Formula VIII, R^1 is



and m is 0.

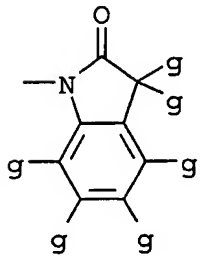
In one embodiment of the compounds of Formula VIII, R^1 is



m is 0, and R^7 is $-(CH_2)_n$-aryl.

In one embodiment of the compounds of Formula VIII, R^7 is $-(CH_2)_n$-phenyl.

In a preferred embodiment of the compounds of Formula VIII, Z is



and each g is hydrogen.

Preferred hydroxamate ICE inhibitor compounds include:

3-Benzyloxycarbonyl-amino-4-oxo-5-phenylacetylaminooxy-pentanoic acid;

3-Benzyloxycarbonylamino-4-oxo-5-(2-oxo-pyrrolidin-1-yloxy)-pentanoic acid;

3-Benzyloxycarbonylamino-5-(3,5-dioxo-10-oxa-4-aza-tricyclo[5.2.1.$0^{2,6}$]dec-8-en-4-yloxy)-4-oxo-pentanoic acid;

3-Benzyloxycarbonylamino-5-(2-oxo-2,3-dihydro-indol-1-yloxy)-4-oxo-pentanoic acid;

3-Benzyloxycarbonylamino-5-(7-methoxycarbonylmethyl-2-oxo-octahydro-indol-1-yloxy)-4-oxo-pentanoic acid;

3-Benzyloxycarbonylamino-4-oxo-5-(2-oxo-octahydro-indol-1-yloxy)-pentanoic acid;

3-[2-(2-Benzyloxycarbonylamino-3-methyl-butyrylamino)-propionylamino]-5-(7-methoxycarbonylmethyl-2-oxo-octahydro-indol-1-yloxy)-4-oxo-pentanoic acid;

3-[2-(2-benzyloxycarbonylamino-3-methyl-butyrylamino)-propionylamino]-4-oxo-5-(2-oxo-2,3-dihydro-indol-1-yloxy)-pentanoic acid;

3-Benzyloxycarbonylamino-5-(2,5-dioxo-pyrrolidin-1-yloxy)-4-oxo-pentanoic acid;

3-Benzyloxycarbonylamino-4-oxo-5-(2,2,3-trimethyl-5-oxo-pyrrolidin-1-yloxy)-pentanoic acid;

3-Benzyloxycarbonylamino-5-(1,3-dioxo-octahydro-isoindol-2-yloxy)-4-oxo-pentanoic acid;

3-Benzyloxycarbonylamino-5-(1,3-dioxo-1,3-dihydro-isoindol-2-yloxy)-4-oxo-pentanoic acid;

3-Benzyloxycarbonylamino-5-[3-(4-bromo-phenyl)-2,5-dioxo-2,5-dihydro-pyrrol-1-yloxy]-4-oxo-pentanoic acid;

3-Benzyloxycarbonylamino-5-(3,5-dioxo-4-aza-tricyclo[5.2.1.$0^{2,6}$]dec-8-en-4-yloxy)-4-oxo-pentanoic acid;

3-Benzyloxycarbonylamino-5-(2,4-dioxo-3-aza-spiro[5.5]undec-3-yloxy)-4-oxo-pentanoic acid;

5-(2-Biphenyl-4-yl-5-oxo-pyrrolidin-1-yloxy)-4-oxo-3-(2-propenyl-penta-2,4-dienyloxycarbonyl amino)-pentanoic acid;

5-Benzoylaminooxy-3-benzyloxycarbonylamino-4-oxo-pentanoic acid;

3-Benzyloxycarbonylamino-4-oxo-5-(3-phenyl-propionyl-aminooxy)-pentanoic acid;

3-Benzyloxycarbonylamino-5-(2-naphthalen-1-yl-acetyl-aminooxy)-4-oxo-pentanoic acid;

3-Benzyloxycarbonylamino-5-(3-naphthalen-1-yl-propionylaminooxy)-4-oxo-pentanoic acid;

3-Benzyloxycarbonylamino-5-[methyl-(3-phenyl-propionyl)-aminooxy]-4-oxo-pentanoic acid;

5-(Benzoyl-methyl-aminooxy)-3-benzyloxycarbonylamino-4-oxo-pentanoic acid;

3-Benzyloxycarbonylamino-5-[methyl-(3-naphthalen-1-yl-propionyl)-aminooxy]-4-oxo-pentanoic acid;

3-Benzyloxycarbonylamino-5-[methyl-(naphthalen-1-yl-acetyl)-aminooxy]-4-oxo-pentanoic acid;

3-Benzyloxycarbonylamino-5-[benzyl-(3-phenyl-propionyl)-aminooxy]-4-oxo-pentanoic acid;

5-[Benzyl-(3-naphthalen-1-yl-propionyl)-aminooxy]-3-benzyloxycarbonylamino-4-21-pentanoic acid;

5-(3-Benzyl-2-oxo-pyrrolidin-1-yloxy)-4-oxo-3-(2-propenyl-penta-2,4-dienyloxycarbonylamino)-pentanoic acid;

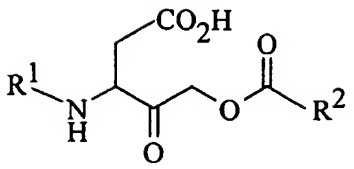
5-(3-Methyl-2-oxo-pyrrolidin-1-yloxy)-4-oxo-3-(2-propenyl-penta-2,4-dienyloxycarbonylamino)-pentanoic acid;

3-Benzyloxycarbonylamino-4-oxo-5-[methyl-(phenylacetyl)-aminooxy]-pentanoic acid; or

3-Benzyloxycarbonylamino-4-oxo-5-(1-oxo-1,3-dihydro-isoindol-2-yloxy)-pentanoic acid.

Hydroxamate ICE inhibitors as described above can be prepared, for example, as described in Example 12.

Still other types of ICE inhibitors that can be used in the invention include aspartate ester inhibitors, including compounds having the formula IX:



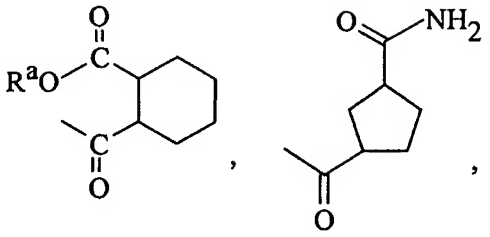
IX

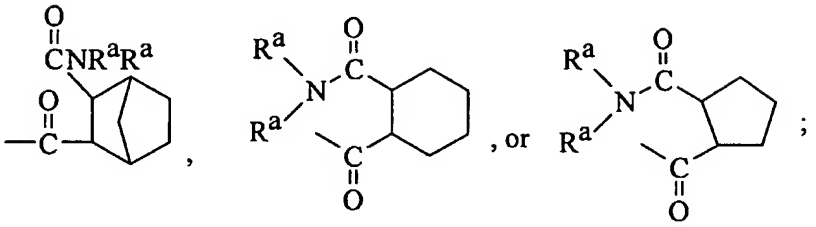
wherein R^1 is $R^3OC(O)$-,

R^3CO-,

R^3SO_2-,

$R^5N(R^a)CHR^6CO$-





each R^a is independently hydrogen, C_1-C_6 alkyl, or -$(CH_2)_n$ aryl;

R^2 is -$(CRR)_n$-aryl,

-$(CRR)_n$-X-aryl,

-$(CRR)_n$-heteroaryl,

-$(CRR)_n$-X-heteroaryl,

-$(CRR)_n$-(substituted-heteroaryl),

-$(CRR)_n$-(substituted-aryl),

-$(CRR)_n$-X-(substituted-aryl),

-$(CRR)_n$-aryl-aryl,

-$(CRR)_n$-aryl-heteroaryl,

-$(CRR)_n$-aryl-$(CH_2)_n$-aryl,

-$(CRR)_n$-$CH(aryl)_2$,

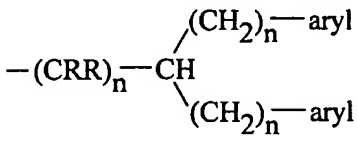
-$(CRR)_n$-cycloalkyl,

-$(CRR)_n$-X-cycloalkyl,

-$(CRR)_n$-heterocycle,

-$(CRR)_n$-X-heterocycle,

-$(CRR)_n$ substituted heterocycle,



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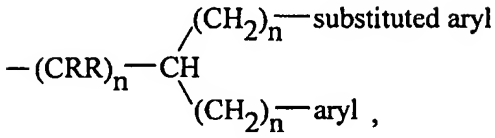

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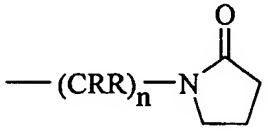

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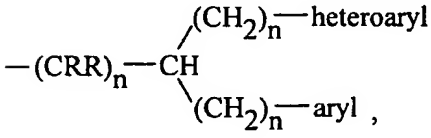

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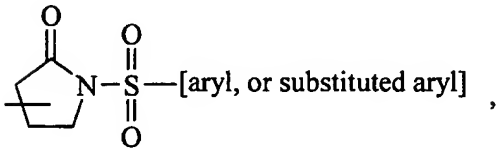

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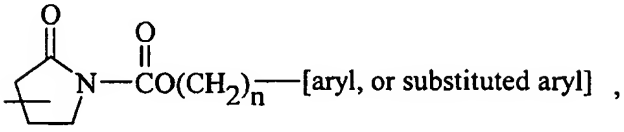

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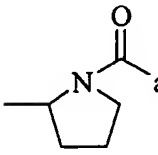

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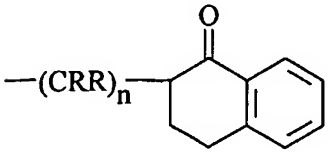

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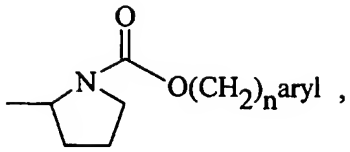
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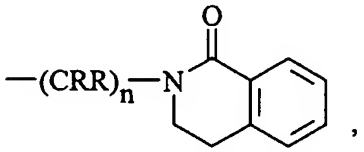
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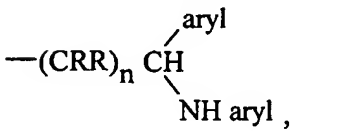
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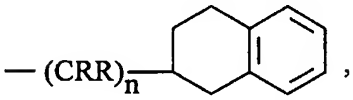
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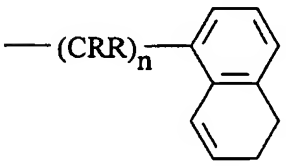
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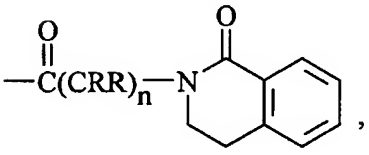
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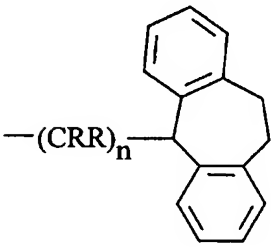
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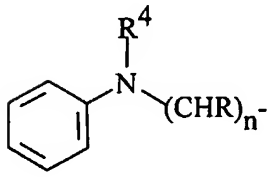
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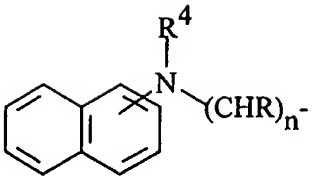

;

each R is independently hydrogen, C_1-C_6 alkyl, halogen or hydroxy;

X is O or S;

R^3 is C_1-C_6 alkyl,

aryl,

heteroaryl,

-$(CHR)_n$-aryl,

-$(CHR)_n$-heteroaryl,

-$(CHR)_n$-substituted heteroaryl,

-$(CHR)_n$-substituted aryl,

-$(CRR)_nC(O)ORa$,

-$(CRR)_nS(CH_2)_n$-aryl,

cycloalkyl,

substituted cycloalkyl,

heterocycle,

substituted heterocycle,

-(CRR)nC(O)NRaRa

-$(CRR)_n$-SO_2-$(CH_2)_n$ aryl,

-$(CRR)_n$-SO_2-C_1-C_6 alkyl,

J-$CH_2C(R^a)H$,

-$CHR^6C(O)$-heteroaryl,

-$(CRR)_nS(CH_2)_nC(O)OR^a$,

-(CRR)n-SO_2-$(CH_2)_nC(O)ORa$,

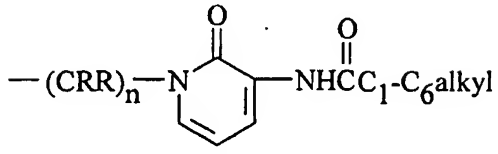
-$(CRR)_nS(CH_2)_n$-aryl,

-$(CRR)_n$-SO_2-$(CH_2)_n$-aryl,

-$(CRR)_nSC(O)C_1$-C_6 alkyl,

-$(CRR)_nS(O)(CH_2)_n$ aryl,

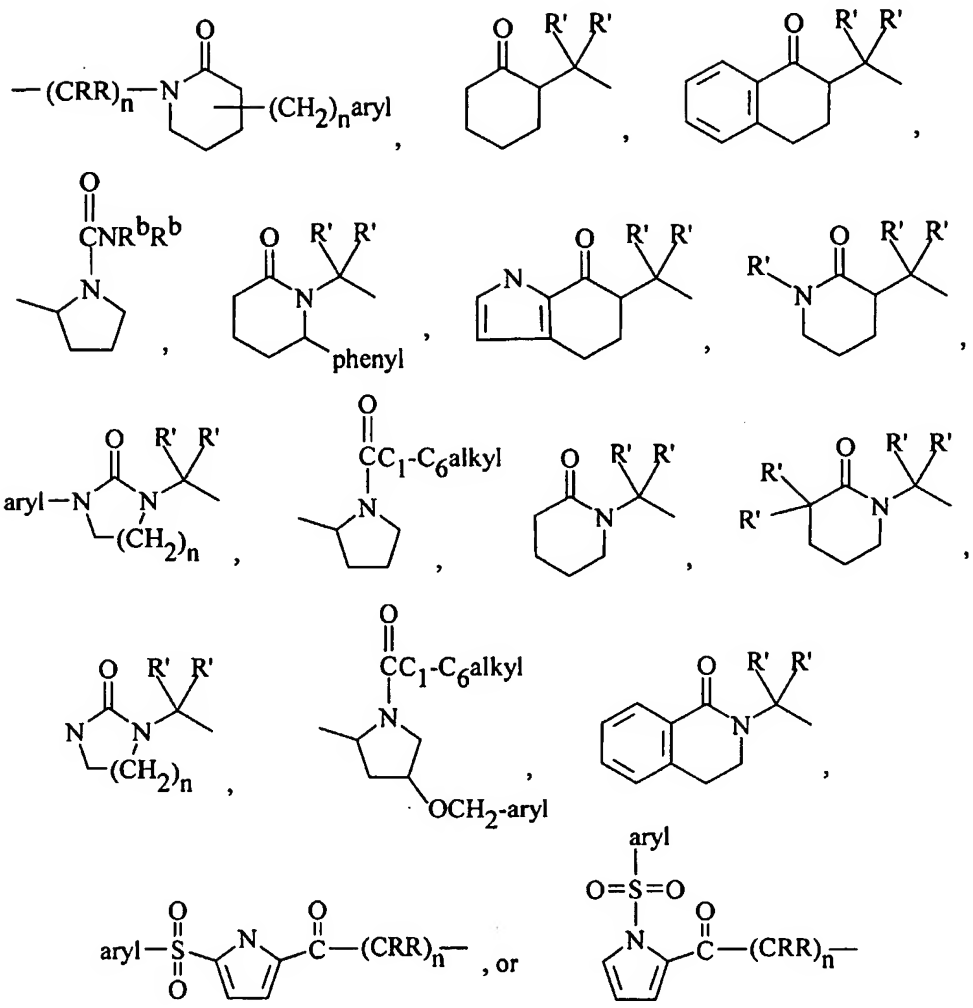
-$(CRR)_nS(O)(CH_2)_nCO_2R^a$,



,

-$(CH_2)_nNHC(O)C_1$-C_6 alkyl,

-$(CH_2)_nC(O)NR^bR^b$,



each R′ is independently C_1-C_6 alkyl,

C_1-C_6 alkylaryl,

aryl, or

hydrogen;

each J is independently

$-CO_2R^b$,

$-CONR^bR^b$,

$-SO_2NR^bR^b$, or

$-SO_2R^b$;

each R^b is independently hydrogen, C_1-C_6 alkyl, aryl, substituted aryl; arylalkyl, heteroarylalkyl, substituted arylalkyl, or substituted heteroarylalkyl;

R^4 is hydrogen,

C_1-C_6 alkyl,

$CH_3OC(O)$-,

-phenyl, or

C_1-C_6 alkyl C(O)-;

R^5 is C_1-C_6 alkyl-CO-,

-$(CH_2)_n$ aryl,

C_1-C_6-alkylOC(O)-,

C_1-C_6-alkyl-X-$(CH_2)_n$CO,

C_1-C_6-alkyl-X-$(CH_2)_n$OC(O)-,

-C(O)(CRR)$_n$aryl,

-C(O)NR^aR^a,

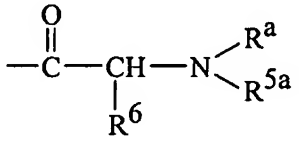
-SO_2-C_1-C_6 alkyl,

-C(O)$(CH_2)_n$C(O)NR^aR^a,

-C(O)O$(CH_2)_n$ aryl,

-C(O)O$(CH_2)_n$ substituted aryl,

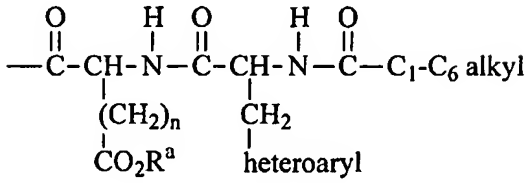
-C(O)(CRR)$_n$NHC(O)O$(CH_2)_n$-aryl,



,

-$(CH_2)_n$X$(CH_2)_n$-aryl,

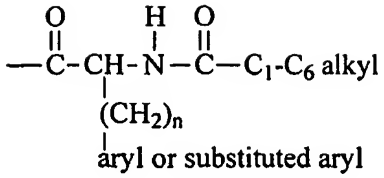
-C_1-C_6 alkyl X-C_1-C_6 alkyl aryl, or



R^{5a} is

$C(O)C_1$-C_6 alkyl,

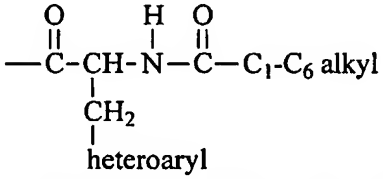
-$C(O)OC_1$-C_6 alkyl,



$C(O)O(CH_2)_n$ aryl,

$C(O)(CH_2)_n$ aryl, or

R^6 is hydrogen,



C_1-C_6 alkyl, -$(CH_2)_n$ aryl, -$(CH_2)_nCO_2R^a$, hydroxyl substituted C_1-C_6 alkyl, or imidazole substituted C_1-C_6 alkyl;

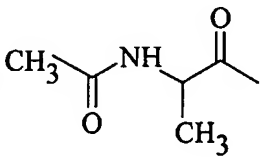
each n is independently 0 to 3, and the pharmaceutically acceptable, salts, esters, amides, and prodrugs thereof.

In one embodiment of the compounds of Formula IX, R^1 is phenyl-CH_2-OC(O)-.

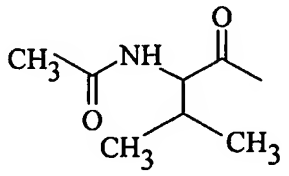
In another embodiment of the compounds of Formula IX, R^1 is phenyl-SO_2-.

In another embodiment of the compounds of Formula IX, R^1 is CH_3-OC(O)-.

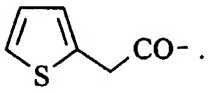
In another embodiment of the compounds of Formula IX, R^1 is phenyl-CH_2CH_2-CO-.

In another embodiment of the compounds of Formula IX, R^1 is  .

In another embodiment of the compounds of Formula IX, R^1 is



In another embodiment of the compounds of Formula IX, R^1 is phenyl-CH_2-CO-.

In another embodiment of the compounds of Formula IX, R^1 is  .

In another embodiment of the compounds of Formula IX, each R^a is hydrogen.

In another embodiment of the compounds of Formula IX, R^2 is -$(CH_2)_n$-phenyl.

In another embodiment of the compounds of Formula IX, R^2 is -$(CH_2)_n$-naphthyl.

In another embodiment of the compounds of Formula IX, R^2 is -$(CH_2)_n$-O-phenyl.

In another embodiment of the compounds of Formula IX, R^2 is -$(CH_2)_n$-O-naphthyl.

In another embodiment of the compounds of Formula IX, R^2 is -$(CH_2)_n$-S-phenyl.

In another embodiment of the compounds of Formula IX, R^2 is-$(CH_2)_n$-CH(phenyl)$_2$..

In another embodiment of the compounds of Formula IX, each R^a is hydrogen; R^1 is benzyloxycarbonyl; R^2 is aryl-X$(CRR)_n$-, aryl-$(CRR)_n$-, heteroaryl-$(CRR)_n$-, or cycloalkyl-$(CRR)_n$-; n is 1, 2, or 3; X is O or S; and R is hydrogen, methyl, or benzyl.

In another embodiment of the compounds of Formula IX, each R^a is hydrogen;

R^1 is benzyloxycarbonyl; and

R^2 is -$(CH_2)_n$-naphthyl,

-$(CH_2)_n$-phenyl,

-$(CH_2)_n$-cycloalkyl,

-$(CH_2)_nO(CH_2)_n$-naphthyl,

-$(CH_2)_nO(CH_2)_n$-phenyl, or

-$(CH_2)_nS(CH_2)_n$-phenyl.

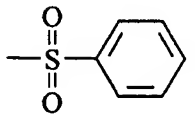
In another embodiment of the compounds of Formula IX, each R^a is hydrogen;

R^1 is benzyloxycarbonyl; and

R^2 is -CH_2-naphthyl.

In another embodiment of the compounds of Formula IX, each R^a is hydrogen;

R^2 is benzyloxycarbonyl,



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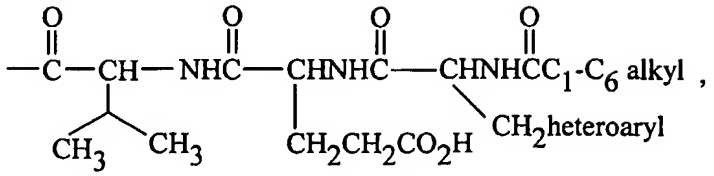

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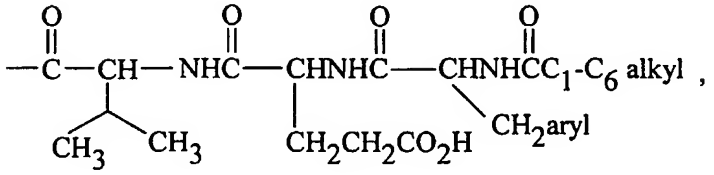

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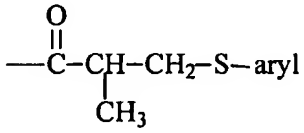
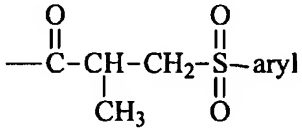
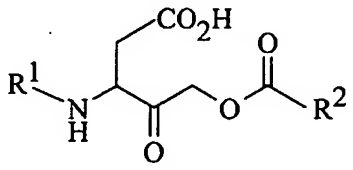




Other aspartate ester ICE inhibitor compounds that can be used in the invention include compounds of the Formula X:



X

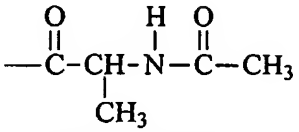
wherein R^1 is

-C(O)OCH_2 phenyl,

-SO_2-phenyl,

-C(O)OCH_3,

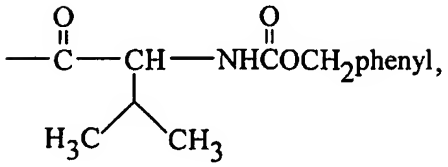
$C(O)CH_2CH_2$ phenyl,

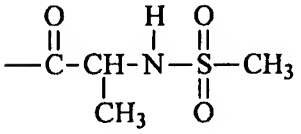


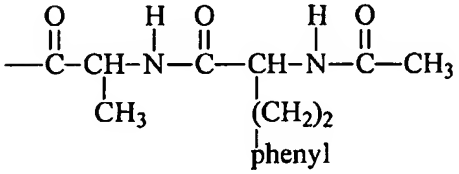
$-C(O)CH_2$ thienyl,

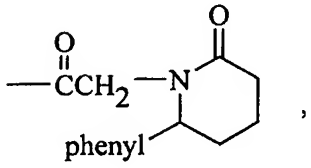
$-C(O)C_1$-C_6 alkyl,

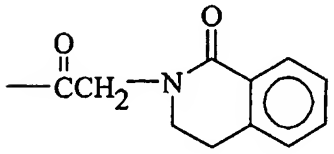
$-(CH_2)_3$ phenyl,

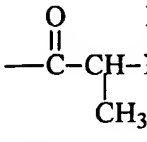


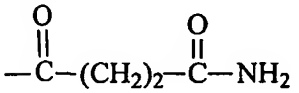


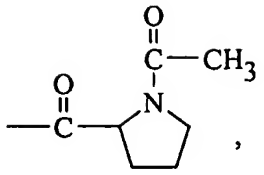












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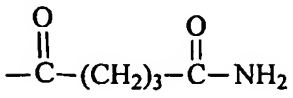

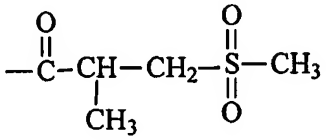

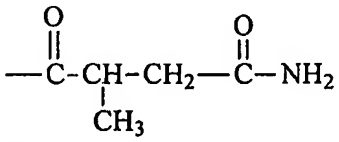

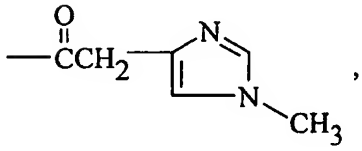

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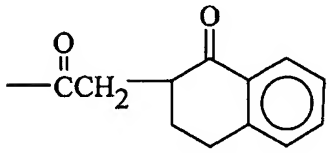

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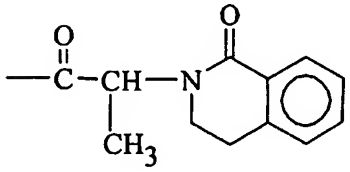

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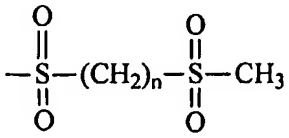

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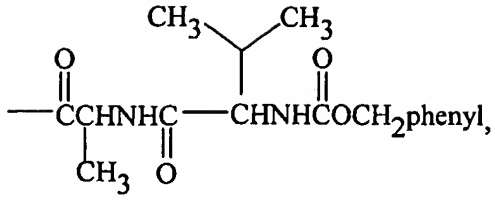

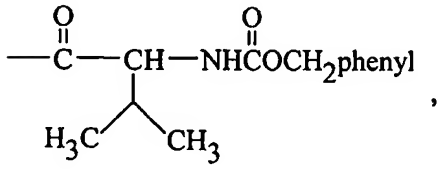

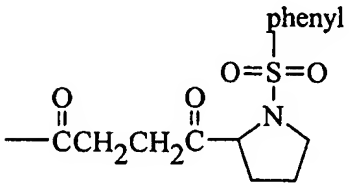

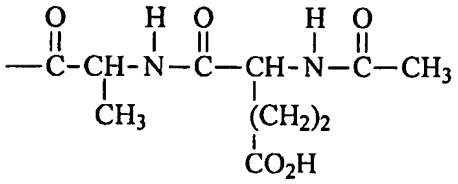

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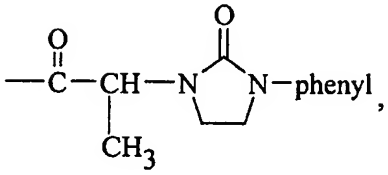

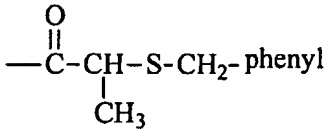

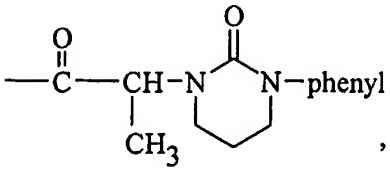

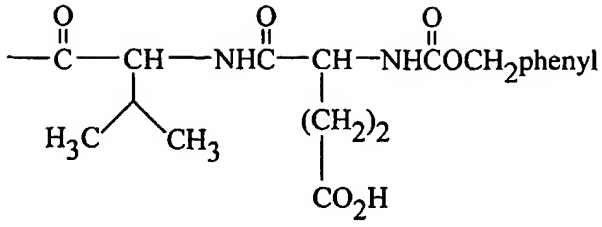

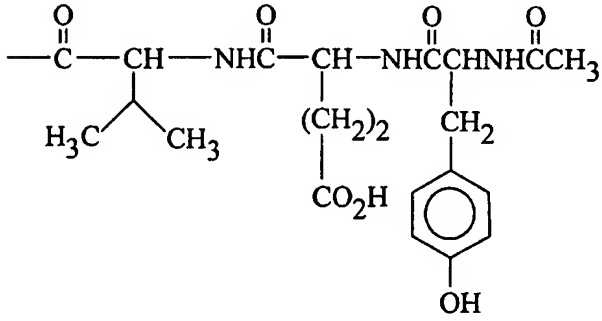

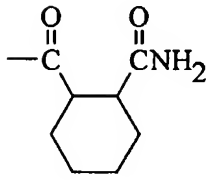

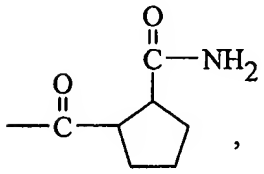

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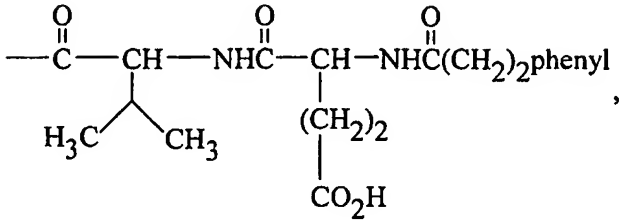

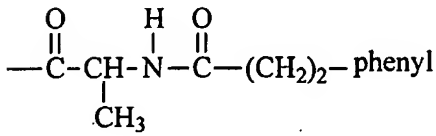

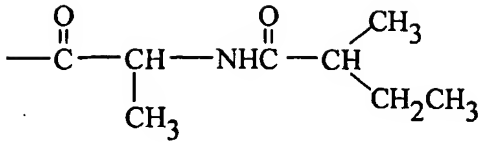

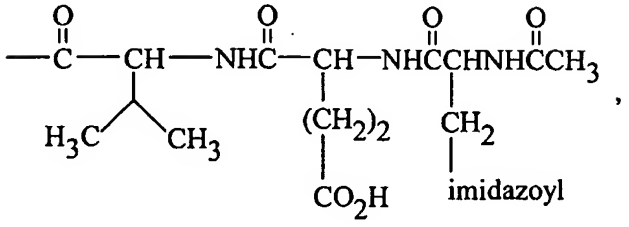

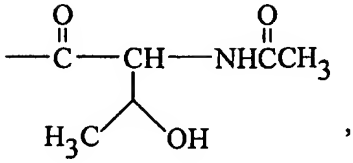

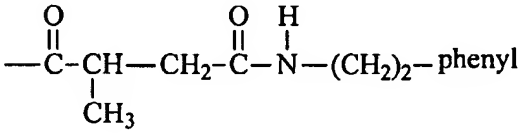

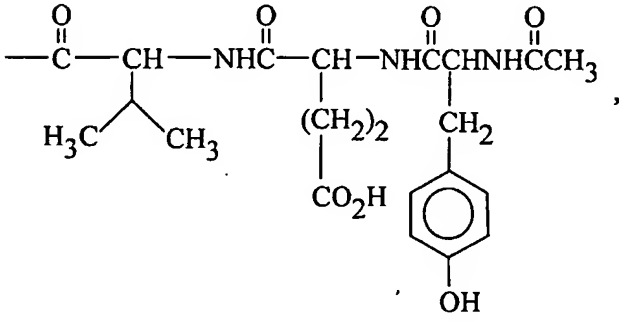

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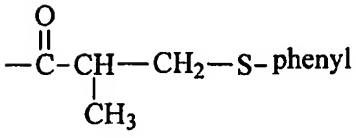

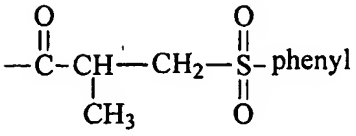

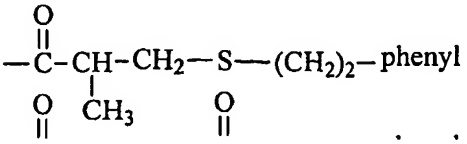

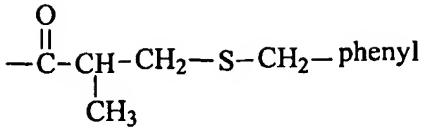

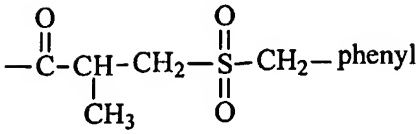

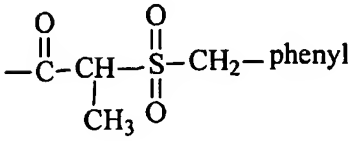

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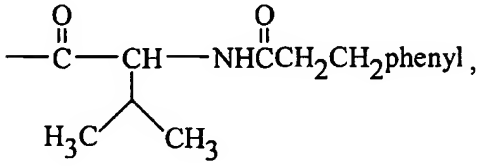
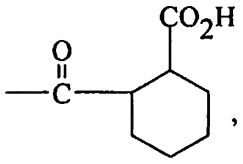
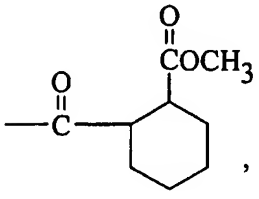
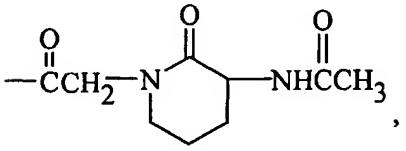
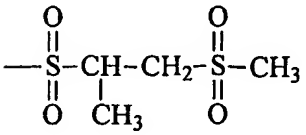
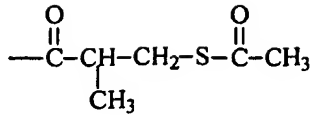


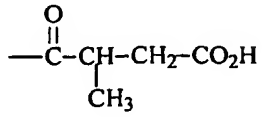


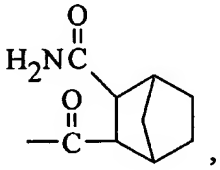


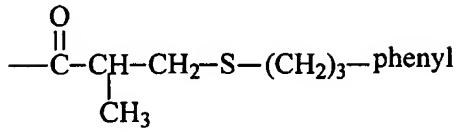


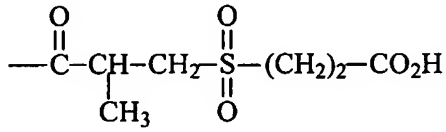


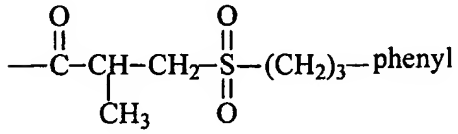






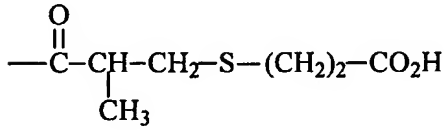




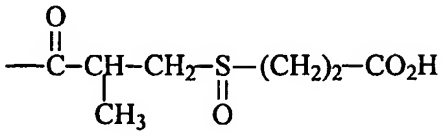


or

R^2 is



-CH_2CH_2 phenyl,



-CH_2 naphthyl,

-CH_2CH_2 cyclohexyl,

-CH_2O naphthyl,

-CH_2O phenyl,

-CH_2S-phenyl,

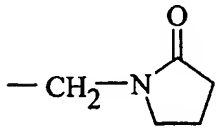
-CH_2-substituted naphthyl,

-$CH_2CH(phenyl)_2$,

-CH_2-imidazole,

-$(CH_2)_3$-phenyl,

-$C(CH_3)H$-naphthyl,



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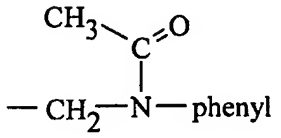
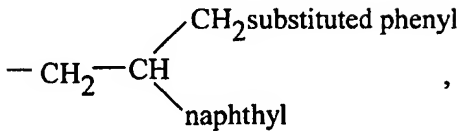


,

-$CH[CH_2phenyl]_2$,

-C(OH)H-naphthyl,

-CH_2-NH phenyl,

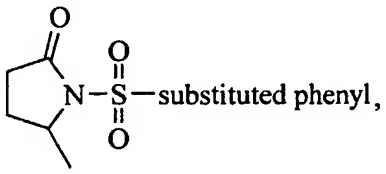


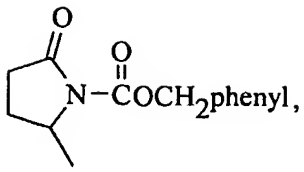
,

-CH_2-naphthyl-phenyl,

-CH_2-fluorenyl,

-CH_2 naphthyl-thienyl,



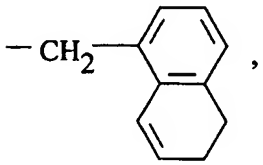


-CH_2-benzofuranyl,

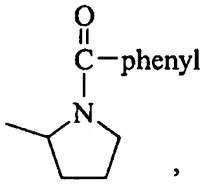
-CH_2-benzothienyl,

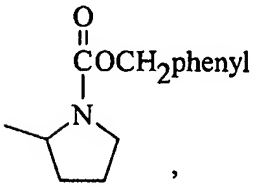
-CH_2-naphthyl-CH_2 phenyl,

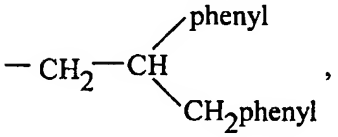
-CH_2-substituted phenyl,

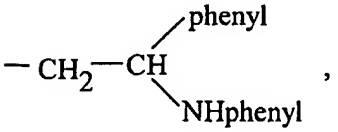


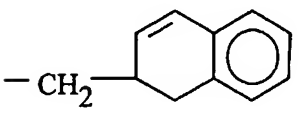
-CH_2-substituted indolyl,

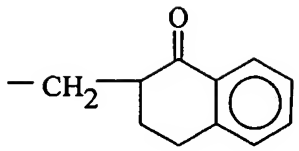








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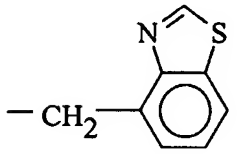
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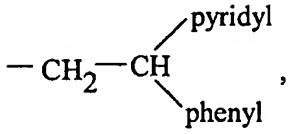
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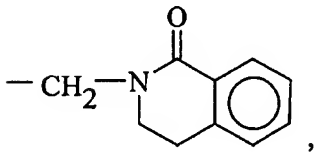
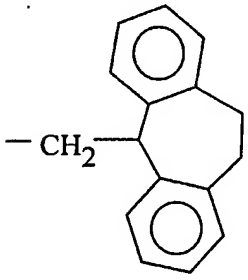

,

-$(CH_2)_2$ pyridyl, or


,

each n is independently 0 to 3, and the pharmaceutically acceptable, salts, esters, amides, and prodrugs thereof.

Preferred aspartate ester ICE inhibitor compounds include the compounds:

3-Benzyloxycarbonylamino-5-(naphthalen-1-yl-acetoxy)-4-oxo-pentanoic acid;

3-Benzyloxycarbonylamino-4-oxo-5-(3-phenyl-propionyloxy)-pentanoic acid;

3-Benzyloxycarbonylamino-5-(3-cyclohexyl-propionyloxy)-4-oxo-pentanoic acid;

3-Benzyloxycarbonylamino-5-[(naphthalene-1-yl-oxy)-acetoxy]-4-oxo-pentanoic acid;

3-Benzyloxycarbonylamino-4-oxo-5-phenoxyacetoxy-pentanoic acid;

3-Benzyloxycarbonylamino-4-oxo-5-phenylsulfanylacetoxy-pentanoic acid;

3-Benzyloxycarbonylamino-5-[(6-methoxy-naphthalene-1-yl)-acetoxy]-4-oxo-pentanoic acid;

3-Benzyloxycarbonylamino-5-(naphthalene-2-yl-acetoxy)-4-oxo-pentanoic acid;

3-Benzyloxycarbonylamino-5-(3-naphthalene-2-yl-propionyloxy)-4-oxo-pentanoic acid;

3-Benzyloxycarbonylamino-5-(3,3-diphenyl-propionyloxy)-4-oxo-pentanoic acid;

3-Benzyloxycarbonylamino-5-[(1H-indol-3-yl)-acetoxy]-4-oxo-pentanoic acid;

3-Benzyloxycarbonylamino-5-(indol-1-yl-acetoxy)-4-oxo-pentanoic acid;

3-Benzyloxycarbonylamino-5-(2-naphthalene-1-yl-propionyloxy)-4-oxo-pentanoic acid;

3-Benzyloxycarbonylamino-4-oxo-5-[(2-oxo-pyrrolidin-1-yl)-acetoxy]-pentanoic acid;

5-[(Acetyl-phenyl-amino)-acetoxy]-3-benzyloxycarbonyl-amino-4-oxo-pentanoic acid;

3-Benzyloxycarbonylamino-5-(2-benzyl-3-phenyl-propionyloxy)-4-oxo-pentanoic acid;

3-Benzyloxycarbonylamino-5-(hydroxy-naphthalene-1-yl-acetoxy)-4-oxo-pentanoic acid;

3-Benzyloxycarbonylamino-4-oxo-5-[(phenyl-amino)-acetoxy]-pentanoic acid;

3-Benzyloxycarbonylamino-5-[(6-hydroxy-naphthalene-1-yl)-acetoxy]-4-oxo-pentanoic acid;

3-Benzyloxycarbonylamino-5-[3-(4-hydroxy-phenyl)-2-naphthalene-1-yl-propionyloxy)-4-oxo-pentanoic acid;

(S)-3-Benzyloxycarbonylamino-4-oxo-5-phenylacetoxy-pentanoic acid;

(S)-3-Benzyloxycarbonylamino-4-oxo-5-(4-phenyl-butyryloxy)-pentanoic acid;

3-Benzyloxycarbonylamino-4-oxo-5-[(4-phenyl-naphthalen-1-yl)-acetoxy]-pentanoic acid;

3-Benzyloxycarbonylamino-5-[(4-methyl-naphthalen-1-yl)-acetoxy]-4-oxo-pentanoic acid;

3-Benzyloxycarbonylamino-4-oxo-5-[(4-thiophen-2-yl-naphthalen-1-yl)-acetoxy]-pentanoic acid;

3-Benzyloxycarbonylamino-5-[(4-fluoro-naphthalen-1-yl)-acetoxy]-4-oxo-pentanoic acid;

3-Benzyloxycarbonylamino-5-[(2-methyl-naphthalen-1-yl)-acetoxy]-4-oxo-pentanoic acid;

3-Benzyloxycarbonylamino-5-[(2-fluoro-naphthalen-1-yl)-acetoxy]-4-oxo-pentanoic acid;

5-(Benzofuran-4-yl-acetoxy)-3-benzyloxycarbonylamino-4-oxo-pentanoic acid;

5-(Benzo[b]thiophen-7-yl-acetoxy)-3-benzyloxycarbonylamino-4-oxo-pentanoic acid;

5-(Benzo[b]thiophen-4-yl-acetoxy)-3-benzyloxycarbonylamino-4-oxo-pentanoic acid;

5-[(4-Benzyl-naphthalen-1-yl)-acetoxy]-3-benzyloxycarbonylamino-4-oxo-pentanoic acid;

3-Benzyloxycarbonylamino-5-[(3,4-dihydro-naphthalen-1-yl)-acetoxy]-4-oxo-pentanoic acid;

3-Benzyloxycarbonylamino-5-[(5-bromo-1H-indol-3-yl)-acetoxy]-4-oxo-pentanoic acid;

3-Benzyloxycarbonylamino-5-(3,4-diphenyl-butyryloxy)-4-oxo-pentanoic acid;

3-Benzyloxycarbonylamino-4-oxo-5-(3-phenyl-3-phenylamino-propionyloxy)-pentanoic acid;

3-Benzyloxycarbonylamino-4-oxo-5-[(1,2,3,4-tetrahydro-naphthalen-2-yl)-acetoxy]-pentanoic acid;

3-Benzyloxycarbonylamino-5-[(1-methanesulfonyl-piperidin-4-yl)-acetoxy]-4-oxo-pentanoic acid;

3-Benzyloxycarbonylamino-4-oxo-5-[(2,3,5,6-tetramethyl-phenyl)-acetoxy]-pentanoic acid;

5-(Benzothiazol-4-yl-acetoxy)-3-benzyloxycarbonylamino-4-oxo-pentanoic acid;

5-(Benzofuran-3-yl-acetoxy)-3-benzyloxycarbonylamino-4-oxo-pentanoic acid;

5-(Benzo[b]thiophen-3-yl-acetoxy)-3-benzyloxycarbonylamino-4-oxo-pentanoic acid;

3-Benzyloxycarbonylamino-4-oxo-5-(3-phenyl-3-pyridin-2-yl-propionyloxy)-pentanoic acid;

3-Benzyloxycarbonylamino-5-[(2,3-dichloro-phenyl)-acetoxy]-4-oxo-pentanoic acid;

3-Benzyloxycarbonylamino-5-[(5-methyl-naphthalen-1-yl)-acetoxy]-4-oxo-pentanoic acid;

3-Benzyloxycarbonylamino-5-[(2-iodo-phenyl)-acetoxy]-4-oxo-pentanoic acid;

3-Benzyloxycarbonylamino-4-oxo-5-(3-pyridin-3-yl-propionyloxy)-pentanoic acid;

3-Benzyloxycarbonylamino-5-[(5-methoxy-naphthalen-1-yl)-acetoxy]-4-oxo-pentanoic acid;

3-Benzyloxycarbonylamino-5-[(8-methyl-naphthalen-1-yl)-acetoxy]-4-oxo-pentanoic acid;

3-Benzyloxycarbonylamino-5-[(9H-fluoren-9-yl)-acetoxy]-4-oxo-pentanoic acid;

3-Benzyloxycarbonylamino-5-[(10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5-yl)-acetoxy]-4-oxo-pentanoic acid;

5-Oxo-1-(toluene-4-sulfonyl)-pyrrolidine-2-carboxylic acid 3-benzyloxycarbonylamino-4-carboxy-2-oxo-butyl ester;

5-Oxo-pyrrolidine-1,2-dicarboxylic acid 1-benzyl ester 2-(3-benzyloxycarbonylamino-4-carboxy-2-oxo-butyl) ester;

1-Benzoyl-pyrrolidine-2-carboxylic acid 3-benzyloxycarbonylamino-4-carboxy-2-oxo-butyl ester;

Pyrrolidine-1,2-dicarboxylic acid 1-benzyl ester 2-(3-benzyloxycarbonylamino-4-carboxy-2-oxo-butyl) ester;

3-Benzyloxycarbonylamino-5-(2-benzyl-3-phenyl-propionyloxy)-4-oxo-pentanoic acid;

3-Benzyloxycarbonylamino-5-[(5-cyano-naphthalen-1-yl)-acetoxy]-4-oxo-pentanoic acid;

3-Benzyloxycarbonylamino-4-oxo-5-(3-phenyl-3-pyridin-3-yl-propionyloxy)-pentanoic acid;

3-Benzyloxycarbonylamino-4-oxo-5-(3-phenyl-3-pyridin-4-yl-propionyloxy)-pentanoic acid; and

3-Benzyloxycarbonylamino-4-oxo-5-[(1-oxo-3,4-dihydro-1H-isoquinolin-2-yl)-acetoxy]-pentanoic acid.

3-Benzenesulfonylamino-5-(naphthalen-1-yl-acetoxy)-4-oxo-pentanoic acid;

3-Methoxycarbonylamino-5-(naphthalene-1-yl-acetoxy)-4-oxo-pentanoic acid;

5-(Naphthalene-1-yl-acetoxy)-4-oxo-3-(3-phenyl-propionylamino)-pentanoic acid;

3-Methoxycarbonylamino-4-oxo-5-phenoxyacetoxy-pentanoic acid; and

3-(2-Methanesulfonyl-1-methyl-ethylsulfanylamino)-5-(naphthalen-1-yl-acetoxy)-4-oxo-pentanoic acid.

[S-(R*,R*)]-3-(2-Acetylamino-propionylamino)-5-(naphthalene-1-yl-acetoxy)-4-oxo-pentanoic acid;

5-(Naphthalen-1-yl-acetoxy)-4-oxo-3-[(thiophene-3-carbonyl)-amino]-pentanoic acid;

3-[(Furan-3-carbonyl)-amino]-5-(naphthalen-1-yl-acetoxy)-4-oxo-pentanoic acid;

5-(Naphthalen-1-yl-acetoxy)-4-oxo-3-[2-(4-phenyl-butyrylamino)-propylamino]-pentanoic acid;

3-(2-Methanesulfonylamino-propionylamino)-5-(naphthalen-1-yl-acetoxy)-4-oxo-pentanoic acid;

3-[2-(2-Acetylamino-4-phenyl-butyrylamino)-propionylamino]-5-(naphthalen-1-yl-acetoxy)-4-oxo-pentanoic acid;

3-(2-Acetylamino-butyrylamino)-5-(naphthalen-1-yl-acetoxy)-4-oxo-pentanoic acid;

3-[2-(4-Carbamoyl-butyrylamino)-propionylamino]-5-(naphthalen-1-yl-acetoxy)-4-oxo-pentanoic acid;

3-(2-Benzyloxycarbonylamino-propionylamino)-5-(naphthalen-1-yl-acetoxy)-4-oxo-pentanoic acid;

5-(Naphthalen-1-yl-acetoxy)-4-oxo-3-(2-ureido-propionylamino)-pentanoic acid;

3-(2-Acetylamino-propionylamino)-5-(naphthalen-1-yl-acetoxy)-4-oxo-pentanoic acid;

3-[(1-Acetyl-pyrrolidine-2-carbonyl)-amino]-5-(naphthalen-1-yl-acetoxy)-4-oxo-pentanoic acid;

3-(2-Methyl-3-oxo-3-thiophen-2-yl-propionylamino)-5-(naphthalen-1-yl-acetoxy)-4-oxo-pentanoic acid;

3-(2-Acetylamino-acetylamino)-5-(naphthalen-1-yl-acetoxy)-4-oxo-pentanoic acid;

3-(2-Acetylamino-propionylamino)-5-(3,3-diphenyl-propionyloxy)-4-oxo-pentanoic acid;

3-[2-(2-Acetylamino-4-carboxy-butyrylamino)-propionylamino]-5-(naphthalen-1-yl-acetoxy)-4-oxo-pentanoic acid;

5-(Naphthalen-1-yl-acetoxy)-4-oxo-3-[2-(3-phenyl-propionylamino)-propionylamino]-pentanoic acid;

3-[2-(3-Methyl-butyrylamino)-propionylamino]-5-(naphthalen-1-yl-acetoxy)-4-oxo-pentanoic acid;

3-[(1-Acetyl-4-benzyloxy-pyrrolidine-2-carbonyl)-amino]-5-(naphthalen-1-yl-acetoxy)-4-oxo-pentanoic acid;

3-(4-Carbamoyl-butyrylamino)-5-(naphthalen-1-yl-acetoxy)-4-oxo-pentanoic acid; and

3-[2-(1-Methyl-1H-imidazol-4-yl)-acetylamino]-5-(naphthalen-1-yl-acetoxy)-4-oxo-pentanoic acid.

(S)-5-(Naphthalene-1-yl-acetoxy)-4-oxo-3-phenylacetylamino-pentanoic acid;

(S)-5-(Naphthalene-1-yl-acetoxy)-4-oxo-3-(2-thiophene-2-yl-acetylamino)-pentanoic acid;

3-[(2-Carbamoyl-cyclopentanecarbonyl)-amino]-5-(naphthalen-1-yl-acetoxy)-4-oxo-pentanoic acid;

3-[(3-Carbamoyl-bicyclo[2.2.1]heptane-2-carbonyl)-amino]-5-(naphthalen-1-yl-acetoxy)-4-oxo-pentanoic acid;

3-(3-Methanesulfonyl-2-methyl-propionylamino)-5-(naphthalen-1-yl-acetoxy)-4-oxo-pentanoic acid;

3-(3-Benzenesulfonyl-2-methyl-propionylamino)-5-(naphthalen-1-yl-acetoxy)-4-oxo-pentanoic acid;

3-Butyrylamino-5-(naphthalen-2-yl-acetoxy)-4-oxo-pentanoic acid;

3-Acetylamino-5-(naphthalen-1-yl-acetoxy)-4-oxo-pentanoic acid;

3-(3-Methanesulfonyl-2-methyl-propionylamino)-5-(naphthalen-1-yl-acetoxy)-4-oxo-pentanoic acid;

3-(3-Methyl-butyrylamino)-5-(naphthalen-1-yl-acetoxy)-4-oxo-pentanoic acid;

3-(3-Carbamoyl-propionylamino)-5-(naphthalen-1-yl-acetoxy)-4-oxo-pentanoic acid;

[S-(R*,R*)]-3-(3-Acetylsulfanyl-2-methyl-propionylamino)-5-(naphthalen-1-yl-acetoxy)-4-oxo-pentanoic acid; and

trans-3-[(3-Carbamoyl-cyclopentanecarbonyl)-amino]-5-(naphthalen-1-yl-acetoxy)-4-oxo-pentanoic acid.

3-(1,2,3,4-tetrahydro-1-oxo-isoquinoline-2-yl)-acetamino-5-(naphthalene-1-yl acetoxy)-4-oxo-pentanoic acid;

3-(2-Methyl-3-phenethylcarbamoyl-propionylamino)-5-(naphthalen-1-yl-acetoxy)-4-oxo-pentanoic acid;

5-(Naphthalen-2-yl-acetoxy)-4-oxo-3-[2-(2-oxo-6-phenyl-piperidin-1-yl)-acetylamino]-pentanoic acid;

5-(Naphthalen-1-yl-acetoxy)-4-oxo-3-[2-(2-oxo-6-phenyl-piperidin-1-yl)-acetylamino]-pentanoic acid;

3-[3-Methyl-2-(3-phenyl-propionylamino)-butyrylamino]-4-oxo-5-[(1-oxo-1,2,3,4-tetrahydro-naphthalen-2-yl)-acetoxy]-pentanoic acid;

5-(Naphthalen-2-yl-acetoxy)-4-oxo-3-[2-(1-oxo-3,4-dihydro-1H-isoquinolin-2-yl)-acetylamino]-pentanoic acid;

5-(2-Benzyl-3-phenyl-propionyloxy)-4-oxo-3-[2-(1-oxo-3,4-dihydro-1H-isoquinolin-2-yl)-acetylamino]-pentanoic acid;

5-(2-Benzyl-3-phenyl-propionyloxy)-4-oxo-3-[2-(2-oxo-6-phenyl-piperidin-1-yl)-acetylamino]-pentanoic acid;

5-(Naphthalen-1-yl-acetoxy)-4-oxo-3-[2-(1-oxo-1,2,3,4-tetrahydro-naphthalen-2-yl)-acetylamino]-pentanoic acid;

5-(Naphthalen-1-yl-acetoxy)-4-oxo-3-[2-(1-oxo-3,4-dihydro-1H-isoquinolin-2-yl)-propionylamino]-pentanoic acid;

5-(Naphthalen-2-yl-acetoxy)-4-oxo-3-[2-(1-oxo-3,4-dihydro-1H-isoquinolin-2-yl)-propionylamino]-pentanoic acid;

3-[4-(1-Benzenesulfonyl-1H-pyrrol-2-yl)-4-oxo-butyrylamino]-5-(naphthalen-1-yl-acetoxy)-4-oxo-pentanoic acid;

5-(2-Benzyl-3-phenyl-propionyloxy)-4-oxo-3-[2-(1-oxo-1,2,3,4-tetrahydro-naphthalen-2-yl)-acetylamino]-pentanoic acid;

5-(2-Benzyl-3-phenyl-propionyloxy)-4-oxo-3-[2-(1-oxo-3,4-dihydro-1H-isoquinolin-2-yl)-propionylamino]-pentanoic acid;

4-Oxo-3-[2-(1-oxo-3,4-dihydro-1H-isoquinolin-2-yl)-propionylamino]-5-[(1-oxo-1,2,3,4-tetrahydro-naphthalen-2-yl)-acetoxy]-pentanoic acid;

3-[4-(1-Benzenesulfonyl-1H-pyrrol-2-yl)-4-oxo-butyrylamino]-5-(2-benzyl-3-phenyl-propionyloxy)-4-oxo-pentanoic acid;

4-Oxo-5-[(1-oxo-1,2,3,4-tetrahydro-naphthalen-2-yl)-acetoxy]-3-[2-(1-oxo-1,2,3,4-tetrahydro-naphthalen-2-yl)-acetylamino]-pentanoic acid;

5-(Naphthalen-1-yl-acetoxy)-4-oxo-3-[2-(2-oxo-3-phenyl-imidazolidin-1-yl)-propionylamino]-pentanoic acid;

5-(Naphthalen-1-yl-acetoxy)-4-oxo-3-[2-(2-oxo-3-phenyl-tetrahydro-pyrimidin-1-yl)-propionylamino]-pentanoic acid;

5-(Naphthalen-1-yl-acetoxy)-4-oxo-3-[2-(2-oxo-3-phenyl-tetrahydro-pyrimidin-1-yl)-acetylamino]-pentanoic acid;

3-(2-Acetylamino-3-methyl-butyrylamino)-5-(naphthalen-1-yl-acetoxy)-4-oxo-pentanoic acid;

3-(2-Acetylamino-3-methyl-butyrylamino)-5-(2-benzyl-3-phenyl-propionyloxy)-4-oxo-pentanoic acid;

3-(2-Acetylamino-3-methyl-butyrylamino)-5-(3-benzyl-4-phenyl-butyryloxy)-4-oxo-pentanoic acid;

3-(2-Acetylamino-3-methyl-butyrylamino)-5-(4-benzyl-5-phenyl-pentanoyloxy)-4-oxo-pentanoic acid;

3-(2-Acetylamino-3-methyl-butyrylamino)-4-oxo-5-[(1-oxo-1,2,3,4-tetrahydro-naphthalen-2-yl)-acetoxy]-pentanoic acid;

5-(3-Benzyl-4-phenyl-butyryloxy)-3-[3-methyl-2-(3-phenyl-propionylamino)-butyrylamino]-4-oxo-pentanoic acid;

3-[2-(3-Acetylamino-2-oxo-2H-pyridin-1-yl)-acetylamino]-5-(3,3-diphenyl-propionyloxy)-4-oxo-pentanoic acid; and

3-[2-(3-Acetylamino-2-oxo-2H-pyridin-1-yl)-acetylamino]-5-(2-benzyl-3-phenyl-propionyloxy)-4-oxo-pentanoic acid.

3-[2-(2-Benzyloxycarbonylamino-4-carboxy-butyrylamino)-3-methyl-butyrylamino]-5-(naphthalen-1-yl-acetoxy)-4-oxo-pentanoic acid;

3-[2-(2-Benzyloxycarbonylamino-3-methyl-butyrylamino)-propionylamino]-5-(naphthalen-1-yl-acetoxy)-4-oxo-pentanoic acid;

3-(2-Acetylamino-3-methyl-butyrylamino)-5-(naphthalen-1-yl-acetoxy)-4-oxo-pentanoic acid;

3-[2-(2-Benzyloxycarbonylamino-3-methyl-butyrylamino)-propionylamino]-5-(3,3-diphenyl-propionyloxy)-4-oxo-pentanoic acid;

3-[2-(2-Benzyloxycarbonylamino-3-methyl-butyrylamino)-propionylamino]-5-(2-benzyl-3-phenyl-propionyloxy)-4-oxo-pentanoic acid;

3-[2-(2-Benzyloxycarbonylamino-3-methyl-butyrylamino)-propionylamino]-5-(naphthalen-1-yl-acetoxy)-4-oxo-pentanoic acid;

5-(2-Benzyl-3-phenyl-propionyloxy)-3-{2-[4-carboxy-2-(3-phenyl-propionylamino)-butyrylamino]-3-methyl-butyrylamino}-4-oxo-pentanoic acid;

3-(2-Benzyloxycarbonylamino-3-methyl-butyrylamino)-5-(3,3-diphenyl-propionyloxy)-4-oxo-pentanoic acid;

3-(2-Acetylamino-3-hydroxy-butyrylamino)-5-(naphthalen-1-yl-acetoxy)-4-oxo-pentanoic acid;

3-(2-Acetylamino-3-hydroxy-butyrylamino)-5-(3,3-diphenyl-propionyloxy)-4-oxo-pentanoic acid;

3-(2-{2-[2-Acetylamino-3-(1H-indol-3-yl)-propionylamino]-4-carboxy-butyrylamino}-3-methyl-butyrylamino)-5-(2-benzyl-3-phenyl-propionyloxy)-4-oxo-pentanoic acid; and

5-(3,3-Diphenyl-propionyloxy)-4-oxo-3-[2-(4-phenyl-butyrylamino)-propionylamino]-pentanoic acid.

3-(2-{2-[2-Acetylamino-3-(1H-indol-3-yl)-propionylamino]-4-carboxy-butyrylamino}-3-methyl-butyrylamino)-5-(naphthalen-1-yl-acetoxy)-4-oxo-pentanoic acid; and

3-(2-{2-[2-Acetylamino-3-(4-hydroxy-phenyl)-propionylamino]-4-carboxy-butyrylamino}-3-methyl-butyrylamino)-5-(naphthalen-1-yl-acetoxy)-4-oxo-pentanoic acid.

3-[(2-Carboxy-cyclohexanecarbonyl)-amino]-5-(naphthalen-1-yl-acetoxy)-4-oxo-pentanoic acid;

3-[(2-Methoxycarbonyl-cyclohexanecarbonyl)-amino]-5-(naphthalen-1-yl-acetoxy)-4-oxo-pentanoic acid; and

3-[(2-Carbamoyl-cyclohexanecarbonyl)-amino]-5-(naphthalen-1-yl-acetoxy)-4-oxo-pentanoic acid.

3-(3-Benzylsulfanyl-2-methyl-propionylamino)-5-(naphthalen-1-yl-acetoxy)-4-oxo-pentanoic acid;

3-(2-Methyl-3-phenylmethanesulfonyl-propionylamino)-5-(naphthalen-1-yl-acetoxy)-4-oxo-pentanoic acid;

3-[3-(2-Carboxy-ethanesulfanyl)-2-methyl-propionylamino]-5-(naphthalen-1-yl-acetoxy)-4-oxo-pentanoic acid;

5-(2-Benzyl-3-phenyl-propionyloxy)-3-[3-(2-carboxy-ethanesulfonyl)-2-methyl-propionylamino]-4-oxo-pentanoic acid;

5-(2-Benzyl-3-phenyl-propionyloxy)-3-[3-(3-carboxy-propane-1-sulfinyl)-2-methyl-propionylamino]-4-oxo-pentanoic acid;

5-(Naphthalen-1-yl-acetoxy)-4-oxo-3-(2-phenylmethanesulfanyl-propionylamino)-pentanoic acid;

3-(2-Methyl-3-phenylsulfanyl-propionylamino)-5-(naphthalen-1-yl-acetoxy)-4-oxo-pentanoic acid;

5-(2-Benzyl-3-phenyl-propionyloxy)-3-(2-methyl-3-phenylsulfanyl-propionylamino)-4-oxo-pentanoic acid;

3-(2-Methyl-3-phenethylsulfanyl-propionylamino)-5-(naphthalen-1-yl-acetoxy)-4-oxo-pentanoic acid;

5-(2-Benzyl-3-phenyl-propionyloxy)-3-(2-methyl-3-phenethylsulfanyl-propionylamino)-4-oxo-pentanoic acid;

5-(2-Benzyl-3-phenyl-propionyloxy)-3-(3-benzylsulfanyl-2-methyl-propionylamino)-4-oxo-pentanoic acid;

5-(2-Benzyl-3-phenyl-propionyloxy)-3-(2-benzylsulfanyl-propionylamino)-4-oxo-pentanoic acid;

3-[2-Methyl-3-(3-phenyl-propylsulfanyl)-propionylamino]-5-(naphthalen-1-yl-acetoxy)-4-oxo-pentanoic acid;

3-(3-Benzenesulfonyl-2-methyl-propionylamino)-5-(naphthalen-1-yl-acetoxy)-4-oxo-pentanoic acid;

3-(3-Benzenesulfonyl-2-methyl-propionylamino)-5-(2-benzyl-3-phenyl-propionyloxy)-4-oxo-pentanoic acid;

5-(2-Benzyl-3-phenyl-propionyloxy)-3-[2-methyl-3-(2-phenyl-ethanesulfonyl)-propionylamino]-4-oxo-pentanoic acid;

3-[2-Methyl-3-(2-phenyl-ethanesulfonyl)-propionylamino]-5-(naphthalen-1-yl-acetoxy)-4-oxo-pentanoic acid;

5-(Naphthalen-1-yl-acetoxy)-4-oxo-3-(2-phenylmethanesulfonyl-propionylamino)-pentanoic acid;

5-(2-Benzyl-3-phenyl-propionyloxy)-3-(2-methyl-3-phenylmethanesulfonyl-propionylamino)-4-oxo-pentanoic acid;

5-(2-Benzyl-3-phenyl-propionyloxy)-4-oxo-3-(2-phenylmethanesulfonyl-propionylamino)-pentanoic acid;

3-[2-Methyl-3-(3-phenyl-propane-1-sulfonyl)-propionylamino]-5-(naphthalen-1-yl-acetoxy)-4-oxo-pentanoic acid;

5-(2-Benzyl-3-phenyl-propionyloxy)-3-[2-methyl-3-(3-phenyl-propane-1-sulfonyl)-propionylamino]-4-oxo-pentanoic acid;

5-(2-Benzyl-3-phenyl-propionyloxy)-3-[3-(2-carboxy-ethylsulfanyl)-2-methyl-propionylamino]-4-oxo-pentanoic acid;

3-[3-(3-Carboxy-propylsulfanyl)-2-methyl-propionylamino]-5-(naphthalen-1-yl-acetoxy)-4-oxo-pentanoic acid;

5-(2-Benzyl-3-phenyl-propionyloxy)-3-[3-(3-carboxy-propylsulfanyl)-2-methyl-propionylamino]-4-oxo-pentanoic acid;

3-(3-Carboxymethylsulfanyl-2-methyl-propionylamino)-5-(naphthalen-1-yl-acetoxy)-4-oxo-pentanoic acid;

5-(2-Benzyl-3-phenyl-propionyloxy)-3-(3-carboxymethylsulfanyl-2-methyl-propionylamino)-4-oxo-pentanoic acid;

3-[3-(2-Carboxy-ethanesulfonyl)-2-methyl-propionylamino]-5-(naphthalen-1-yl-acetoxy)-4-oxo-pentanoic acid;

3-[3-(3-Carboxy-propane-1-sulfonyl)-2-methyl-propionylamino]-5-(naphthalen-1-yl-acetoxy)-4-oxo-pentanoic acid;

3-(3-Carboxymethanesulfonyl-2-methyl-propionylamino)-5-(naphthalen-1-yl-acetoxy)-4-oxo-pentanoic acid;

5-(2-Benzyl-3-phenyl-propionyloxy)-3-[3-(3-carboxy-propane-1-sulfonyl)-2-methyl-propionylamino]-4-oxo-pentanoic acid;

5-(2-Benzyl-3-phenyl-propionyloxy)-3-(3-carboxymethanesulfonyl-2-methyl-propionylamino)-4-oxo-pentanoic acid;

3-[3-(3-Carboxy-propane-1-sulfinyl)-2-methyl-propionylamino]-5-(naphthalen-1-yl-acetoxy)-4-oxo-pentanoic acid;

3-[2-Methyl-3-(3-phenyl-propane-1-sulfinyl)-propionylamino]-5-(naphthalen-1-yl-acetoxy)-4-oxo-pentanoic acid; and

5-(2-Benzyl-3-phenyl-propionyloxy)-3-[2-methyl-3-(3-phenyl-propane-1-sulfinyl)-propionylamino]-4-oxo-pentanoic acid.

3-[3-Methyl-2-(phenethylcarbamoyl-methyl)-butyrylamino]-5-(naphthalen-1-yl-acetoxy)-4-oxo-pentanoic acid; and

3-(3-Carboxy-2-methyl-propionylamino)-5-(naphthalen-1-yl-acetoxy)-4-oxo-pentanoic acid.

3-(2-Methyl-3-sulfamoyl-propionylamino)-5-(naphthalen-1-yl-acetoxy)-4-oxo-pentanoic acid.

3-(3-Carbamoyl-2-methyl-propionylamino)-5-(naphthalen-1-yl-acetoxy)-4-oxo-pentanoic acid;

3-(2-Benzyloxycarbonylamino-3-methyl-naphthalen-1-yl-acetoxy)-4-oxo-pentanoic acid;

3-[(2-Carbamoyl-cyclopentanecarbonyl)-amino]-5-(naphthalen-1-yl-acetoxy)-4-oxo-pentanoic acid;

3-[(1-Carbamoyl-pyrrolidine-2-carbonyl)-amino]-5-(naphthalen-1-yl-acetoxy)-4-oxo-pentanoic acid;

3-(2-{2-[2-Acetylamino-3-(4-hydroxy-phenyl)-propionylamino]-4-carboxy-butyrylamino}-3-methyl-butyrylamino)-5-(2-benzyl-3-phenyl-propionyloxy)-4-oxo-pentanoic acid;

3-(3-Carbamoyl-2-methyl-propionylamino)-5-(naphthalen-1-yl-acetoxy)-4-oxo-pentanoic acid;

3-(2-Carbamoylmethyl-3-methyl-butyrylamino)-5-(naphthalen-1-yl-acetoxy)-4-oxo-pentanoic acid;

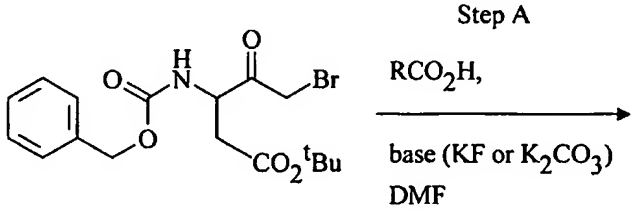
3-(3-Benzyloxy-2-ureido-propionylamino)-5-(naphthalen-1-yl-acetoxy)-4-oxo-pentanoic acid;

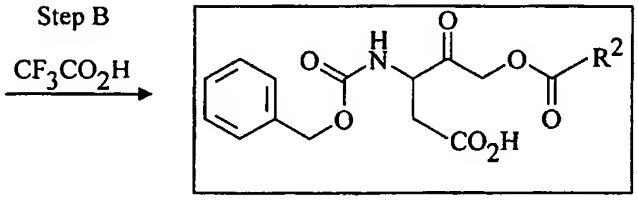
3-[2-(2-Benzyloxycarbonylamino-4-carboxy-butyrylamino)-3-methyl-butyrylamino]-5-(2-benzyl-3-phenyl-propionyloxy)-4-oxo-pentanoic acid;

3-{2-[4-Carboxy-2-(3-phenyl-propionylamino)-butyrylamino]-3-methyl-butyrylamino}-5-(naphthalen-1-yl-acetoxy)-4-oxo-pentanoic acid; and

3-[2-(2-Acetylamino-4-carboxy-butyrylamino)-3-methyl-butyrylamino]-5-(naphthalen-1-yl-acetoxy)-4-oxo-pentanoic acid.

The above-described aspartate ester ICE inhibitors can be made according to the following Schemes 1 through 11: Scheme 1

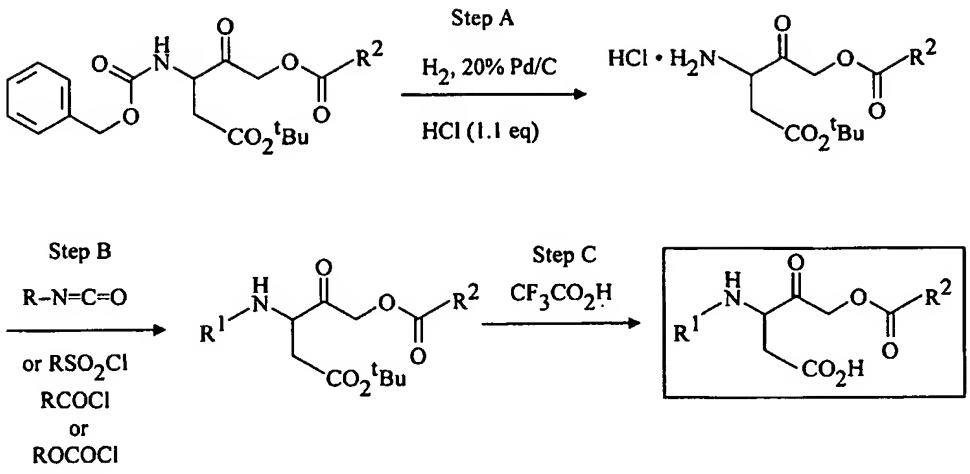




3-Benzyloxycarbonylamino-5-bromo-4-oxo-pentozoic acid tert-butyl ester, also known as Z-Asp(OtBu)-bromomethyl ketone, can be purchased commercially or prepared according to the procedure of Dolle, et al., J. Med. Chem., 1994;37:563-564. This methylbromo ketone is treated with an appropriately substituted carboxylic acid

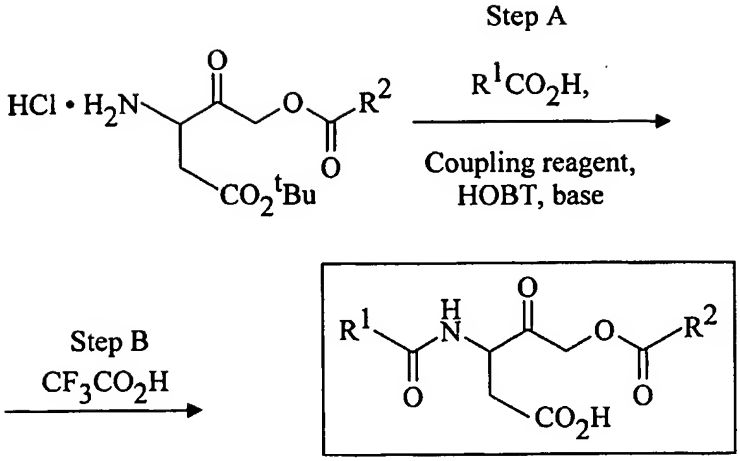
and a base such as potassium fluoride. Alternately, other bases such as potassium carbonate, cesium carbonate, or potassium t-butoxide could be used. The reagents should be mixed in dimethyl formamide (DMF), dimethylacetamide (DMA), dimethyl sulfoxide (DMSO), acetonitrile or other appropriate solvent and stirred at room temperature for 8 to 24 hours. The t-butyl ester protecting group can be removed in acidic media ,preferably trifluoroacetic acid, to produce the carbobenzoxy aspartyl esters shown in Scheme 1.

Scheme 2



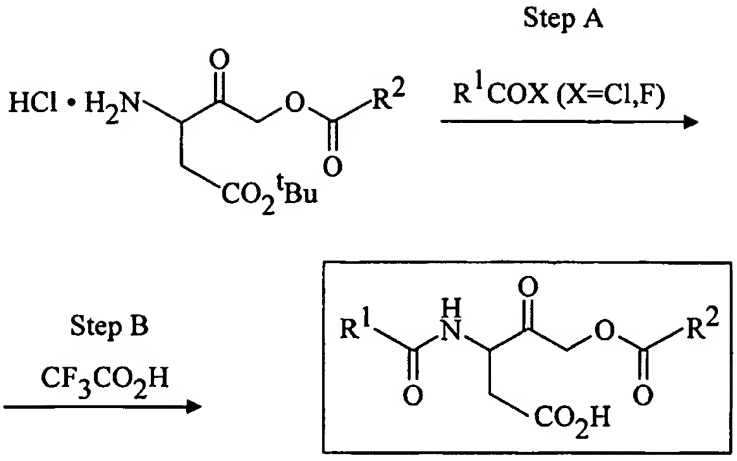
A mixture of an appropriately substituted acyloxymethyl ketone of a carbobenzoxy aspartyl t-butyl ester was hydrogenated with an equivalent of hydrochloric or other acid in the presence of a catalyst such as palladium on carbon to yield the amine salt. The salt can be acylated with an appropriately substituted isocyanate, sulfonyl chloride, chloroformate or phenylpropionyl chloride to afford the N-substituted derivatives. These isocyantes, sulfonyl chlorides, or chloro formates can be purchased commercially or synthesized by methods described in the chemical literature. The t-butyl ester protecting group can be removed in the final step using acidic media, preferably trifluoroacetic acid, to produce the acyloxy methylketone derivatives shown in Scheme 2.

Scheme 3



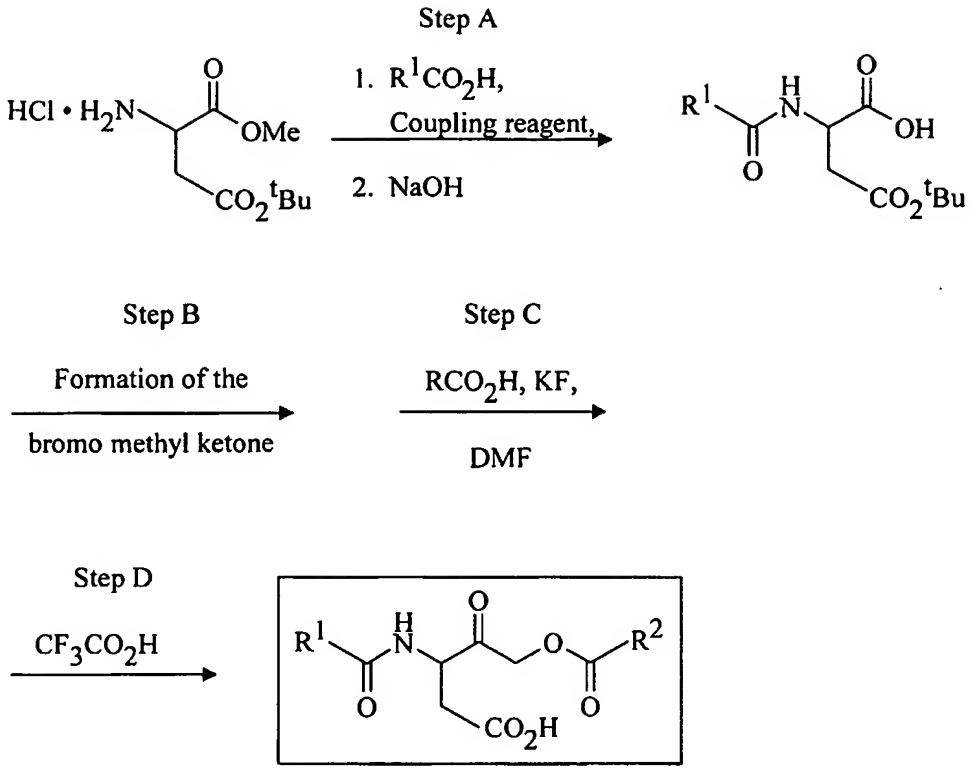
The amine salt of the acyloxymethyl ketone of Z-Asp(Ot-Bu)OH was synthesized and treated with an appropriately substituted carboxylic acid and coupling reagent. The coupling agent may be, but is not limited to, such reagents as 1,3-dicyclohexylcarbodiimide (DCC), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI), 1,1'-carbonyldiimidazole (CDI), 1,1' -carbonylbis(3-methylimidazolium) triflate (CBMIT), isobutylchloroformate, benzotriazol-1-yloxytris(dimethylamino)-phosphonium hexafluorophosphate (BOP), 2-(3,4-dihydro-4-oxo-1,2,3-benzotriazin-3-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TDBTU), and 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU). 1-Hydroxybenzotriazole hydrate should be added to the reaction to improve yield and limit isomerization and base, preferably an amine such as trimethyl amine or methyl morpholine should be added as an acid scavenger. The resulting amide product was treated with acidic media, preferably trifluoroacetic acid, to remove the t-butyl ester and produce the final products as described in Scheme 3.

Scheme 4



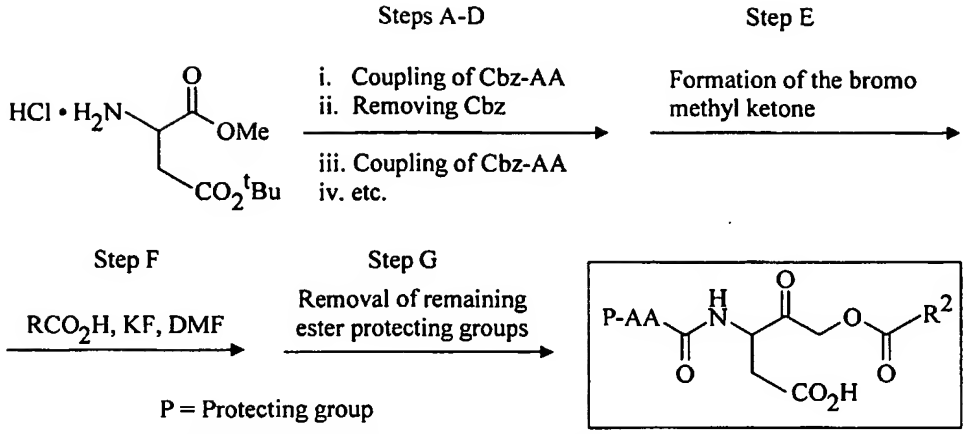
The amine salt of the acyloxymethyl ketone of Cbz-Asp(OtBu)OH was synthesized and treated with an appropriately substituted acid chloride or acid fluoride to generate an amide product. The acid chlorides were purchased commercially or were prepared by treating carboxylic acids with agents such as thionyl chloride, phosphorous tribromide, or oxalyl chloride/DMF. The acid fluorides were prepared by treating a carboxylic acid with cyanuric fluoride. The penultimate amide product was treated with acidic media preferably trifluoroacetic acid to remove the t-butyl ester and afford the final products as described in Scheme 4.

Scheme 5



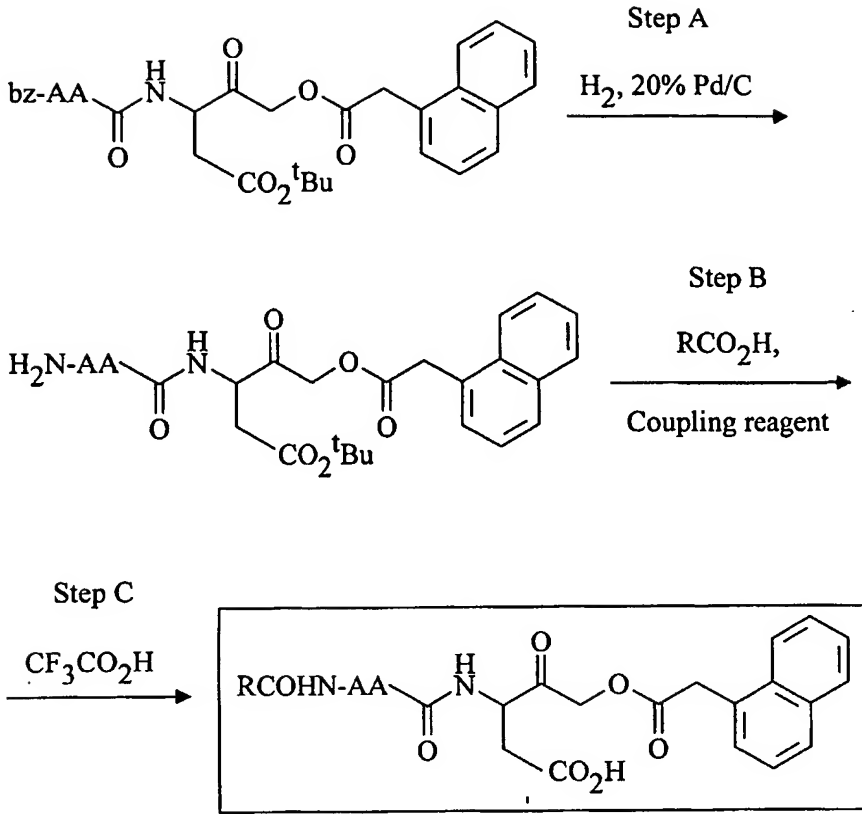
The hydrochloride salt of H-Asp(OtBu)OMe was treated with an appropriately substituted carboxylic acid and coupling reagent. 1-Hydroxybenzotriazole hydrate should be added to the reaction to improve yield and limit isomerization and base, preferably an amine such as trimethyl amine or methyl morpholine should be added as an acid scavenger. The resulting amide product was treated with an alkaline reagent such as sodium hydroxide to hydrolyze the methyl ester to the carboxylic acid. The resulting acid was treated with a chloroformate such as isobutylchloroformate, followed by diazomethane and then hydrobromic acid to afford the methyl bromo ketone. Treatment of the methylbromo ketone with an appropriately substituted carboxylic acid and a base such as potassium fluoride produced the desired acyloxymethyl ketones which were deprotected with trifluoroacetic acid to afford the final compounds as described in Scheme 5.

Scheme 6



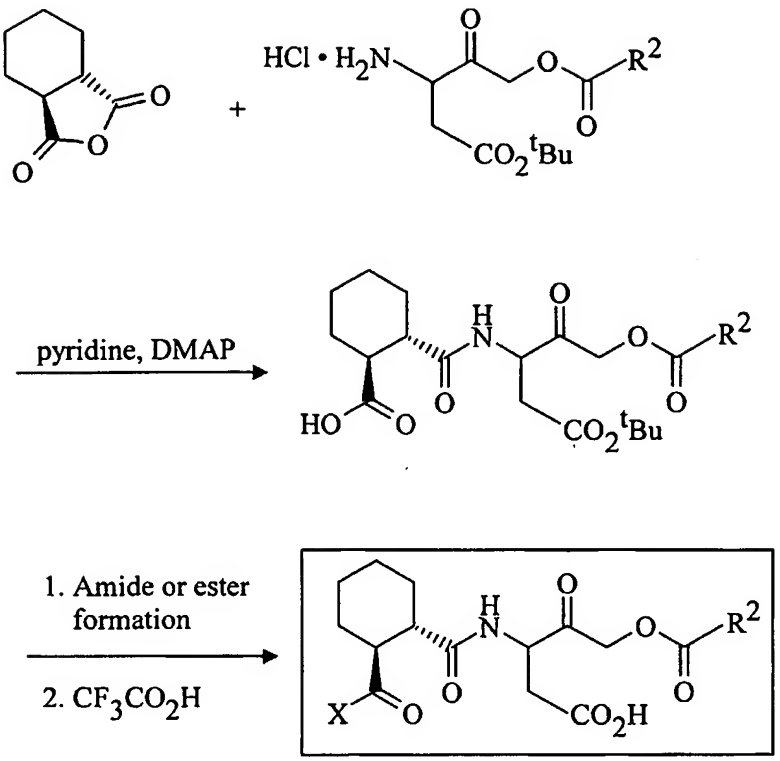
The hydrochloride salt of H-Asp(OtBu)OMe was treated with an appropriately protected amino acid and coupling reagent. 1-Hydroxybenzotriazole hydrate should be added to the reaction to improve yield and limit isomerization and base, preferably an amine such as trimethyl amine or methyl morpholine should be added as an acid scavenger. The resulting amide product was treated with an alkaline reagent such as sodium hydroxide to hydrolyze the methyl ester to the carboxylic acid. The Cbz-amine protecting group was removed using standard catalytic hydrogenation conditions and coupling of another protected amino acid can proceed as described above. This process was repeated until the peptide was the desired length. The resulting peptide product was treated with an alkaline reagent such as sodium hydroxide to hydrolyze the methyl ester to the carboxylic acid. The resulting acid was subsequently treated with a chloroformate such as isobutylchloroformate, followed by diazomethane and then hydrobromic acid to afford the methylbromo ketone. Treatment of the methylbromo ketone with an appropriately substituted carboxylic acid and a base such as potassium fluoride produced the desired acyloxymethyl ketones which were deprotected with trifluoroacetic acid to afford the final compounds as described in Scheme 6.

Scheme 7



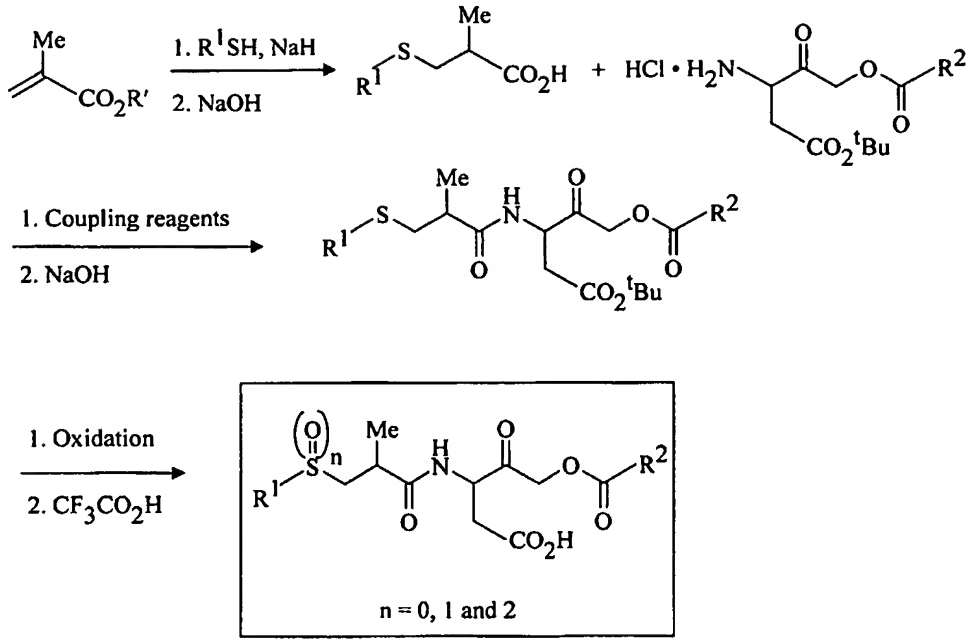
The appropriately substituted acyloxymethyl ketone of a protected amino acid was synthesized. The Cbz-amine protecting group was removed using standard catalytic hydrogenation conditions, and the amine product was treated with an appropriately substituted carboxylic acid and a coupling reagent. 1-Hydroxybenzotriazole hydrate should be added to the reaction to improve yield and limit isomerization and base, preferably an amine such as trimethyl amine or methyl morpholine should be added as an acid scavenger. The penultimate amide product was treated with acidic media preferably trifluoroacetic acid to remove the t-butyl ester and afford the final products as described in Scheme 7.

Scheme 8



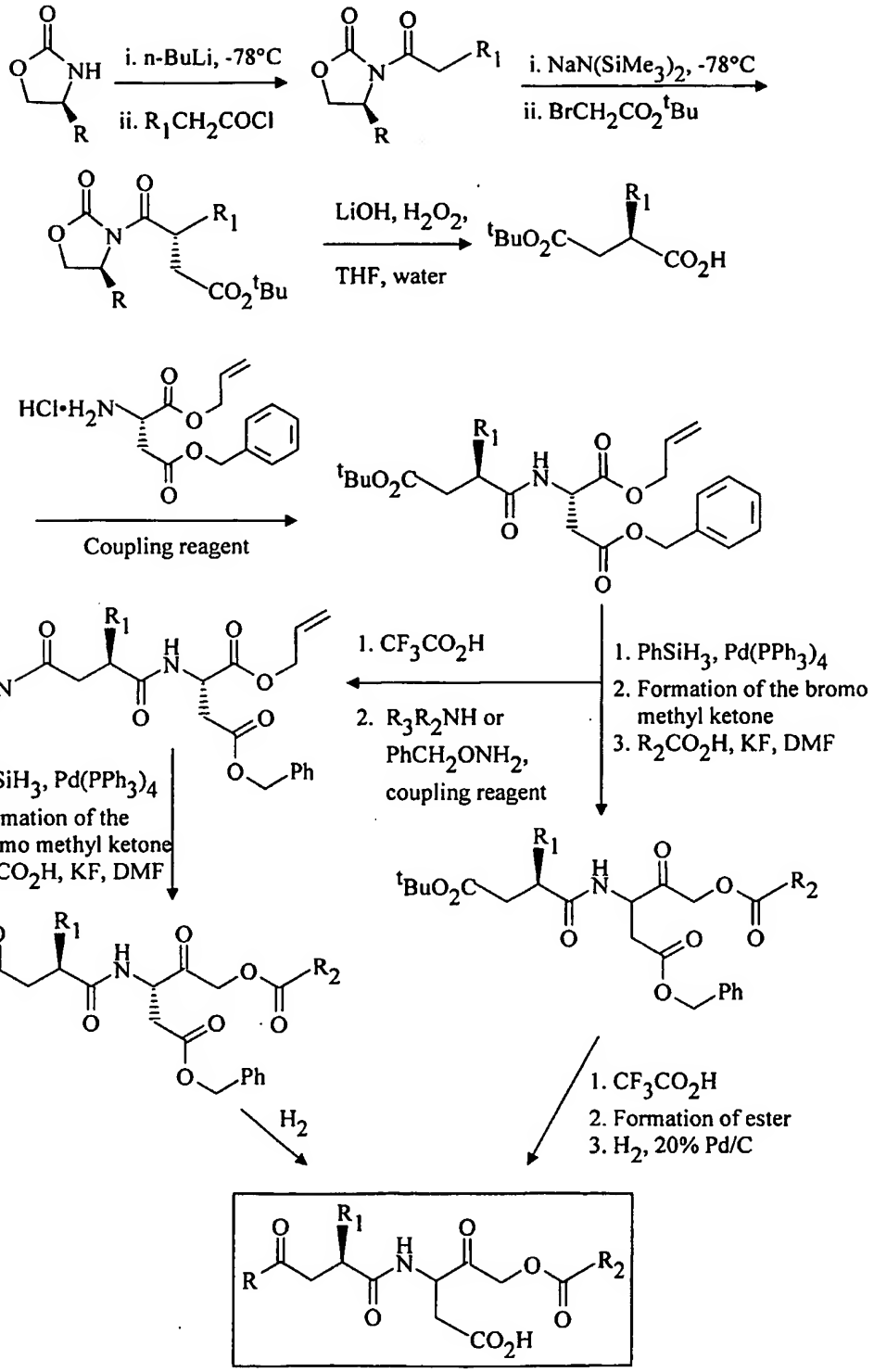
Trans-1,2-cyclohexanedicarboxylic anhydride was treated with the amine salt of an appropriately substituted acyloxymethyl ketone of aspartyl t-butyl ester in the presence of pyridine and 4-dimethylaminopyridine (DMAP) to yield the amide product. The carboxylic acid can be functionalized with appropriately substituted amines or alcohols and standard coupling reagents to afford amide and ester products. The penultimate product was treated with acidic media, preferably trifluoroacetic acid, to remove the t-butyl ester and afford the final products as described in Scheme 8.

Scheme 9



Methyl methacrylate was treated with the anion of an appropriately substituted sulfide to afford the Michael adduct which was hydrolyzed in basic media such as sodium hydroxide to produce the carboxylic acid. This acid was combined with the amine salt of the acyloxymethyl ketone of aspartyl t-butyl ester and a coupling reagent to obtain the amide product. If the sulfide (where n = 0) is the desired product, no oxidation step is employed, and the amide t-butyl ester is deprotected in acidic media, preferably trifluoroacetic acid, to afford the final product. Alternately, if the sulfoxide (n = 1) or sulfone (n = 2) is the final product, the amide intermediate is treated with an oxidizing agent which may be, but is not limited to, m-chloroperbenzoic acid, potassium monoperoxysulfate, or sodium perborate to obtain the desired oxidized product. The t-butyl ester of the penultimate intermediate was deprotected in acidic media, preferably trifluoroacetic acid, to afford the final compounds as described in Scheme 9.

Scheme 10

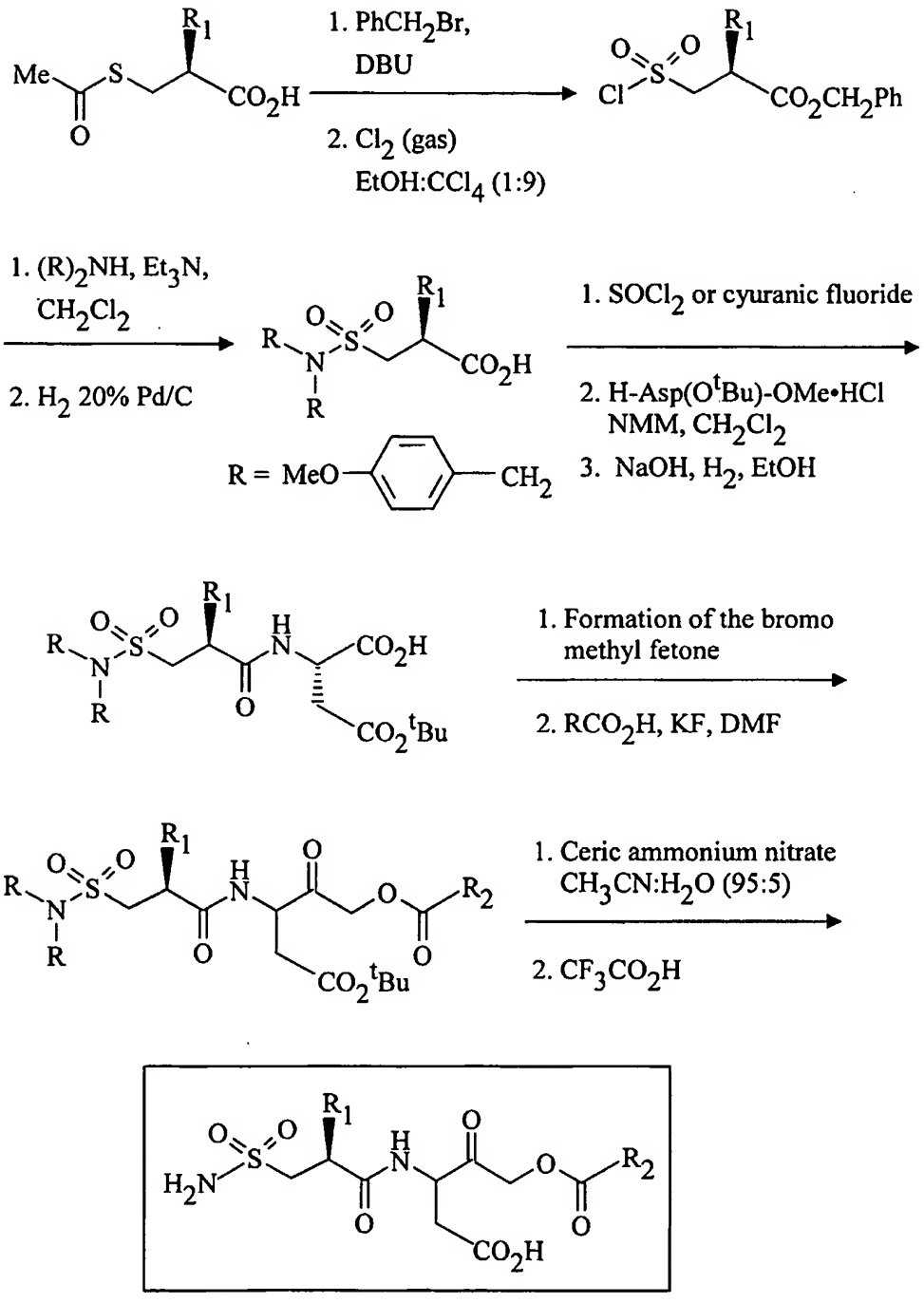

i. n-BuLi, -78°C
ii. R1CH2COCl
i. NaN(SiMe3)2, -78°C
ii. BrCH2CO2tBu
LiOH, H2O2,
THF, water
Coupling reagent
1. CF3CO2H
2. R3R2NH or PhCH2ONH2, coupling reagent
1. PhSiH3, Pd(PPh3)4
2. Formation of the bromo methyl ketone
3. R2CO2H, KF, DMF
1. PhSiH3, Pd(PPh3)4
2. Formation of the bromo methyl ketone
3. R2CO2H, KF, DMF
H2
1. CF3CO2H
2. Formation of ester
3. H2, 20% Pd/C

A 4-substituted-2-oxazolidinone chiral auxiliary as described by Evans, et al., J. Org. Chem., 1985;50:1830 was mixed with a base, such as but not limited to, n-butyl lithium followed by treatment with an appropriately substituted acid chloride or other activated carboxylic acid to afford the N-acylated product. This product was subsequently treated with a base such as, but not limited to, sodium bis(trimethylsilyl)amide and t-butyl bromoacetate to produce the alkylated chiral product. The chiral auxiliary was removed using lithium hydroxide and hydrogen peroxide to obtain the chiral acid. Treatment of the acid with the amine salt of H-Asp(OBz)O-allyl and a coupling reagent afforded the succinyl amide product.

At this stage of the process, the product can be elaborated in one of two ways. First the t-butyl ester was removed in acidic media, preferably trifluoroacetic acid, and the resulting acid was coupled with an appropriately substituted amine in the presence of a coupling reagent to form a new amide product. The allyl ester was removed with phenylsilane and tetrakis(triphenyl-phosphine)palladium or other Pd(0) catalyst to obtain the carboxylic acid, and the acid was converted to the methylbromo ketone and subsequently to the acyloxymethyl ketone. The penultimate intermediate was subjected to catalytic hydrogenation to remove the benzyl ester and afford the final amide products as described in Scheme 10.

Alternatively, in a second route to the final products, the allyl ester is removed using phenylsilane and tetrakistetrakis(triphenylphosphine)palladium or other Pd(0) catalyst to obtain the carboxylic acid. This acid is converted to the methylbromo ketone and subsequently to the acyloxymethyl ketone. Removal of the t-butyl ester of the acyloxymethyl ketone with trifluoroacidic acid and subsequent conversion of the resulting carboxylic acid to the ester resulted in a new ester product. The esterification can be accomplished using a variety of literature techniques which includes but is not limited to treatment of the carboxylic acid with an appropriately substituted alcohol in the presence of a coupling reagent. The penultimate intermediate was subjected to catalytic hydrogenation to remove the benzyl ester and afford the final ester products as described in Scheme 10.

Scheme 11



The appropriately substituted S-acetyl mercapto carboxylic acid was treated with benzyl bromide and 1,8-diazobicyclo[5.4.0]undec-7-ene (DBU) to produce the benzyl ester which was subsequently reacted with chlorine gas to yield the sulfonyl chloride.

The sulfonyl chloride was treated with N,N-bis(p-methoxybenzyl)amine to afford the sulfonamide which was subjected to catalytic hydrogenation to obtain the intermediate carboxylic acid. The acid was activated using cyuranic fluoride which was then mixed with the amine salt of H-Asp(Ot-Bu)OMe to produce the amide product. The methyl ester was hydrolyzed with sodium hydroxide, and the carboxylic acid was elaborated to the acyloxymethyl ketone. The p-methyoxybenzyl protecting groups of the sulfonamide were removed using oxidizing conditions preferably, but not limited to ceric ammonium nitrate, and the t-butyl ester protecting group was removed in acidic media preferably with trifluoroacetic acid to afford the desired sulfonamide products as described in Scheme 11.

As used herein, the term "alkyl" means a straight or branched chain hydrocarbon. Representative examples of alkyl groups are methyl, ethyl, propyl, isopropyl, isobutyl, butyl, tert-butyl, sec-butyl, pentyl, and hexyl.

The term "alkoxy" means an alkyl group attached to an oxygen atom. Representative examples of alkoxy groups include methoxy, ethoxy, tert-butoxy, propoxy, and isobutoxy.

The term "halogen" includes chlorine, fluorine, bromine, and iodine.

The term "aryl" means an aromatic hydrocarbon. Representative examples of aryl groups include phenyl and naphthyl.

The term "heteroatom" includes oxygen, nitrogen, sulfur, and phosphorus.

The term "heteroaryl" means an aryl group wherein one or more carbon atom of the aromatic hydrocarbon has been replaced with a heteroatom. Examples of heteroaryl groups include furan, thiophene, pyrrole, thiazole, pyridine, pyrimidine, pyrazine, benzofuran, indole, coumarin, quinoline, isoquinoline, and naphthyridine.

The term "cycloalkyl" means a cyclic alkyl group. Examples of cycloalkyl groups include cyclopropane, cyclobutane, cyclopentane, and cyclohexane.

The term "heterocycle" means a cycloalkyl group on which one or more carbon atom has been replaced with a heteroatom. Examples of heterocycles include piperazine, morpholine, and piperidine.

The aryl, heteroaryl, or cycloalkyl groups may be substituted with one or more substituents, which can be the same or different. Examples of suitable substituents include alkyl, alkoxy, thioalkoxy, hydroxy, halogen, trifluoromethyl, amino, alkylamino, dialkylamino, $-NO_2$, -CN, $-CO_2H$, $-CO_2$alkyl, $-SO_3H$, -CHO, -COalkyl, $-CONH_2$, -CONH-alkyl, -CONHR^q, $-CON(alkyl)_2$, $-(CH_2)_n-NH_2$, -OH, $-CF_3$, $-OC_1-C_6$alkyl, $-(CH_2)_n$-NH-alkyl, -NHR^q, -NHCOR^q, phenyl, $-(CH_2)_nOH$, $-(CH_2)_nC(O)NH_2$, or $-(CH_2)_nCO_2H$, where n is 1 to 5 and R^q is hydrogen or alkyl.

The symbol "-" means a bond.

Examples of other caspase family inhibitors contemplated for use in the invention include Ich-1 inhibitors such as those described in PCT Publication No. 97/27220.

The term "phosphodiesterase IV inhibitor" is intended to refer to agents that inhibit the activity of the enzyme phosphodiesterase IV. Examples of phosphodiesterase IV inhibitors are known in the art and include 4-arylpyrrolidinones, such as rolipram (see *e.g.,* Sekut, L. *et al.* (1995) *Clin. Exp. Immunol.* 100:126-132), nitraquazone (see *e.g.*, Van Wauwe, J. *et al.* (1995) *Inflamm. Res.* 44:400-405), denbufylline, tibenelast (see *e.g.*, Banner, K.H. *et al.* (1996) *Br. J. Pharmacol.* 119:1255-1261),CP-80633 (see *e.g.*, Cohan, V.L. *et al.* (1996) *J. Pharmacol. Exp. Therap.* 278:1356-1361) and quinazolinediones, such as CP-77059 (see *e.g.,* Sekut, L. *et al.* (1995) *Clin. Exp. Immunol.* 100:126-132).

The term "beta-2 agonist" is intended to refer to agents that stimulate the beta-2 adrenergic receptor. Examples of beta-2 agonists are known in the art and include salmeterol (see *e.g.*, Sekut, L. *et al.* (1995) *Clin. Exp. Immunol.* 99:461-466), fenoterol and isoproterenol (see *e.g.*, Severn, A. *et al.* (1992) *J. Immunol.* 148:3441-3445).

The term "STAT4" is intended to refer to a transcription factor involved in IL-12 responses (see *e.g.*, Thierfelder, W.E. *et al.* (1996) *Nature* 382:171-174; Kaplan, M.H. *et al.* (1996) *Nature* 382:174-177). The term "STAT4 inhibitor" refers to an agent that inhibits the activity of the STAT4 transcription factor such that responses to IL-12 are inhibited.

The term "antibody" as used herein refers to immunoglobulin molecules and immunologically active determinants of immunoglobulin molecules, *i.e.*, molecules that contain an antigen binding site which specifically binds (immunoreacts with) an antigen. The term "antibody" is further intended to include bispecific and chimeric molecules having at least one antigen binding determinant derived from an antibody molecule. Furthermore, although the H and L chains of an Fv fragment are encoded by separate genes, a synthetic linker can be made that enables them to be made as a single protein chain (known as single chain antibody, sAb; Bird *et al.* 1988 *Science* 242:423-426; and Huston *et al.* 1988 *PNAS* 85:5879-5883) by recombinant methods. Such single chain antibodies are also encompassed within the term "antibody", and may be utilized as binding determinants in the design and engineering of a multispecific binding molecule. Advantageously, the antibodies are neutralizing antibodies. The antibodies of the invention are preferably chimeric, humanized, or fully human and may be generated through methods known in the art. For example, the antibodies may be produced recombinantly through the use of a hybridoma or through the use of a transgenic animal, e.g., a mouse. The antibodies of the invention may be specific for agents such as, e.g., IL-12 or IL-18, their receptors, e.g., IL-12 receptor or IL-18 receptor, or their precursors e.g., pro-IL-12, or pro-IL-18. Particularly preferred antibodies included anti-IL-18 monoclonal antibodies, J695, and D2E7 (described in U.S. Patent Appln. Serial No.: 09/125,098).

The term "antibody fragment" as used herein refers to an active fragment of an antibody that retains the ability to bind (immunoreact with) an antigen. Examples of antibody fragments include: a Fab fragment consisting of the V_L, V_H, $C_L$and C_{H1} domains; an Fd fragment consisting of the V_H and C_{H1} domains; an Fv fragment consisting of the V_L and V_H domains of a single arm of an antibody; a dAb fragment (Ward *et al.*, 1989 *Nature* 341:544-546) consisting of a V_H domain; an isolated complementarity determining region (CDR); and an $F(ab')_2$ fragment, a bivalent fragment comprising two Fab' fragments linked by a disulfide bridge at the hinge region. These antibody fragments are obtained using conventional techniques well-known to those with skill in the art, and the fragments are screened for utility in the same manner as are intact antibodies. Examples of preferred antibody fragments include fragments of

chimeric, humanized and human antibodies. Advantageously, the antibody fragments are neutralizing. The antibody fragments of the invention may be specific for agents such as, e.g., IL-12 or IL-18, or their receptors, e.g., IL-12 receptor or IL-18 receptor.

The term "natural or native binding protein" includes proteins native to humans which bind or otherwise interact with IL-18 or IL-12, resulting in a decrease or down regulation of IFN-γ.

The term "engineered binding protein" as used herein is intended to include molecules derived from an antibody or other binding molecule (*e.g.*, a receptor or ligand) that retain a desired binding specificity but that have been engineered by recombinant DNA techniques and/or are expressed using recombinant DNA techniques. Examples of engineered binding proteins include soluble and truncated forms of receptors, dimers of receptors (*e.g.*, p40 IL-12 receptor dimers), and modified or mutated forms of antibodies, ligands or receptors selected using combinatorial libraries (*e.g.*, phage display library techniques).

The term "NK cell antagonist" as used herein is intended to include antibodies, antibody fragments and engineered binding proteins that are capable of depleting NK/NK-like cells when administered to a subject. Examples of NK cell antagonists include anti-asialo-GM1 antibodies and NK1.1 antibodies. Other NK cells antagonists include agents which inhibit factors such as IL-1α, IL-1β and TNF, which induce IFN-γ production from NK cells. Examples of agents include anti-IL-1α antibodies, anti-Iβ antibodies and anti-TNF antibodies (e.g., D2E7). Anti TNF antibodies are described more fully in U.S. Patent Application Serial No.: 09/125,098.

The term "NK/NK-like cells" includes cells of the immune system such as B cells, NK cells, macrophages, and neutrophils. The term "NK/NK-like cells" also includes any cell type which stimulates, regulates, or modulates the production of IFN-γ.

The term "T cell antagonist" includes antibodies, antibody fragments and engineered binding proteins that are capable of depleting T-cells (e.g., $CD4^+CD8^+$ T cells, $CD4^-CD8^-$ T cells),when administered to a subject. Examples of T cell antagonists include anti-T-cell antibodies and antibodies directed to specific T-cell activation

markers. Preferred antibodies include anti-CD-4 antibodies, anti-CD28 antibodies, anti-CD8 antibodies, and anti-CD-7 antibodies.

The terms "steroid resistant disease" and "steroid resistant subject" as used herein are intended to refer to diseases and subjects that do not respond significantly to corticosteroid therapy prior to treatment in accordance with the methods of the invention. Steroid resistance is also referred to as steroid refractoriness.

The term "immunoinflammatory disease or disorder" is intended to include inflammatory diseases and disorders in which immune cells and/or cytokines are involved in the pathophysiology of the disease or disorder. The term "acute inflammatory disorder" is intended to include disorders, and episodes of disorders, characterized by rapid onset of symptoms associated with an inflammatory response and relatively short duration of symptoms, whereas a "chronic inflammatory disorder" is intended to include disorders characterized by the continued presence of symptoms associated with an inflammatory response and ongoing duration of symptoms.

I. Methods of the Invention

In one embodiment, the invention provides a method for modulating responsiveness to a corticosteroid in a subject, comprising administering to the subject:

an agent which antagonizes a target that regulates production of interferon-γ (IFN-γ) in the subject, the agent being administered at a dosage and by a route sufficient to reduce production of IFN-γ in the subject; and

a corticosteroid,

such that responsiveness of the subject to the corticosteroid is modulated as compared to when a corticosteroid alone is administered to the subject.

In one embodiment, the method involves administration of an agent that is an IL-18 antagonist. The IL-18 antagonist is administered to the subject at a dosage and by a route sufficient to inhibit IL-18 activity in the subject. The IL-18 antagonist can act, for example, by inhibiting IL-18 synthesis in the subject, by inhibiting IL-18 cytokine activity in the subject, by inhibiting interaction of IL-18 with an IL-18 receptor or by

inhibiting the activity of an IL-18 receptor. In a preferred embodiment, the IL-18 antagonist is an inhibitor of a caspase family protease. Caspase family proteases, and in particular ICE, process the precursor form of IL-18 to the mature (*i.e.*, active) form (see *e.g.*, Example 4). Accordingly, although not intending to be limited by mechanism, a caspase family protease inhibitor is thought to antagonize IL-18 activity by inhibiting the processing of IL-18 from its precursor form to its mature (*i.e.*, active) form.. A preferred caspase family protease inhibitor for use in the methods of the invention is an ICE inhibitor. Additionally or alternatively, other caspase family proteases that are capable of cleaving precursor IL-18 to mature IL-18 (such as Ich-2 (caspase-4) and $ICE_{rel}III$ (caspase-5)), can be inhibited. Other proteases, such as PR3, which convert precursor IL-18 to active IL-18 can also be inhibited. Chemical agents that can inhibit the activity of ICE and other caspase family proteases are known in the art, including peptidyl derivatives, azaaspartic acid analogs and gamma-pyrone-3-acetic acid (see *e.g.*, U.S. Patent No. 5,411,985, U.S. Patent No. 5,430,128, U.S. Patent No. 5,434,248, U.S. Patent No. 5,565,430, U.S. Patent No. 5,416,013, PCT Publication WO 94/00154, PCT Publication WO 93/16710, PCT Publication WO 93/14777, PCT Publication WO 93/05071, PCT Publication WO 95/35308, European Patent Application EP 547 699 and European Patent Application EP 519 748). Additional suitable inhibitors of ICE and other caspase family inhibitors are disclosed in Example 14, U.S. Application Serial No. 08/700,716 and U.S. Provisional Applications Serial Nos. 60/028,322, 60/028,324, 60/028,313 , 60/028,323, and 60/084,320 (succinamide inhibitors).The exact dosage and regimen for administering an inhibitor of ICE or an ICE-family protease will necessarily depend upon the needs of the subject being treated, the type of treatment, the efficacy of the compound and the degree of disease severity in the subject. However, a nonlimiting example of a dosage range for an inhibitor of ICE and other caspase family proteases is from about 0.05 to about 150 mg/kg body weight/day. In a further embodiment, the method includes administering at least one second agent which inhibits caspase-1 (ICE) independent activation of IL-18. For example, the agent may be a pan-caspase inhibitor, an inhibitor of caspase-4, an inhibitor of caspase-5, or a PR3 inhibitor.

In other embodiments, the IL-18 antagonist is an antibody (e.g., chimeric, humanized or human antibody), antibody fragment (e.g., a chimeric, humanized, or human antibody fragment), a native or natural binding protein (e.g., human IL-18 binding protein), or engineered binding protein that binds IL-18 or an IL-18 receptor. A particularly preferred IL-18 antagonist is an anti-IL-18 monoclonal antibody. The binding agents can be prepared by standard methods known in the art for making poly- and monoclonal antibodies and recombinant binding proteins and are described further in, for example, European Patent Application 692 536, European Patent Application 712 931, PCT Publication WO 97/24441 and PCT Publication WO 97/44468.

In another embodiment, the method of the invention involves administration of an agent that is an IL-12 antagonist. The IL-12 antagonist is administered to the subject at a dosage and by a route sufficient to inhibit IL-12 activity in the subject. The IL-12 antagonist can act, for example, by inhibiting IL-12 synthesis in the subject, by inhibiting IL-12 cytokine activity in the subject, by inhibiting interaction of IL-12 with an IL-12 receptor or by inhibiting the activity of an IL-12 receptor.

In one embodiment, the IL-12 antagonist is an antibody (e.g., a chimeric, human, or humanized antibody), antibody fragment (e.g., a chimeric, human or humanized antibody fragment), a native or natural binding protein, or engineered binding protein that binds IL-12 or IL-12 receptor. Advantageously, the antibody or antibody fragment is neutralizing. A preferred IL-12 antagonist is an anti-IL-12 monoclonal antibody, e.g., a human anti-IL-12 monoclonal antibody such as J695. Such antibodies have been described in the art (see *e.g.*, Chizzonite, R, *et al.* (1991) *J. Immunol.* 147:1548-1556). The ability of anti-IL-12 monoclonal antibodies to inhibit disease responses also has been described in the art (see *e.g.*, Leonard, J.P. *et al.* (1995) *J. Exp. Med.* 181:381-386; Neurath, M.F. *et al.* (1995) *J. Exp. Med.* 182:1281-1290). Another type of IL-12 antagonist is a p40 homodimer (see *e.g.*, Gillessen, S. *et al.* (1995) *Eur. J. Immunol.* 25:200-206; Gately, M.K. *et al.* (1996) *Ann. NY Acad. Sci.* 795:1-12; Ling, P. *et al.* (1995) *J. Immunol.* 154:116-127). Yet another type of IL-12 antagonist is a low affinity form of an IL-12 receptor, as described in European Patent Application EP 638 644 and U.S. Patent No. 5,536,657.

Nonlimiting examples of IL-12 antagonists for use in the methods of the invention include mono- and polyclonal antibodies and fragments thereof, chimeric antibodies and fragments thereof, soluble IL-12 receptors and fragments thereof, reactive peptides or fragments thereof, chemically or genetically modified peptides of IL-12, subunits of IL-12 and fragments thereof, homopolymers of IL-12 subunits and fragments thereof, and small organic molecules designed to inhibit the bioactivity of IL-12 or IL-12 receptors. The preparation of IL-12 antagonists, including: (i) species that bind IL-12 or biologically active fragments thereof, and (ii) species that interfere with the binding of IL-12 to receptors or other binding proteins, have been described in the art (see *e.g.*, PCT Publication WO 95/24918 by Leonard *et al.*, the contents of which are expressly incorporated herein by reference; see also Presky, D.H *et al.* (1995) *Res. Immunol.* 146:439-445).

In another embodiment, an IL-12 antagonist used in the method of the invention is an agent that stimulates cyclic AMP (cAMP) production in cells that produce IL-12. Production of IL-12 has been shown to be inhibited by increased intracellular production of cAMP (see *e.g.*, van der Pouw Kraan *et al.* (1995) *J. Exp. Med.* 181:775-779). Examples of agents that can be used to stimulate intracellular cAMP production include phosphodiesterase IV inhibitors and beta-2 agonist. As demonstrated in Example 3, administration of a phosphodiesterase IV inhibitor in a septic shock model inhibits LPS-induced IL-12 production. Examples of suitable phosphodiesterase IV inhibitors for use in the methods of the invention include rolipram, denbufylline, tibenelast, nitraquazone and CP-80633. Examples of beta-2 agonists for use in the methods of the invention include salmeterol, fenoterol and isoproterenol. The exact dosage and regimen for administering a phosphodiesterase IV inhibitor or a beta-2 agonist will necessarily depend upon the needs of the subject being treated, the type of treatment, the efficacy of the compound and the degree of disease severity in the subject. However, a nonlimiting example of a dosage range for phosphodiesterase IV inhibitors or beta-2 agonists is from about 0.05 to about 150 mg/kg body weight/day. In a preferred embodiment, an agent that stimulates cyclic AMP (cAMP) production (*e.g.*, a phosphodiesterase IV inhibitor or a beta-2 agonist) is administered systemically (*e.g.*, orally or intravenously) to inhibit production of IL-12 systemically by monocytes and macrophages.

In another embodiment, an IL-12 antagonist used in the method of the invention is a STAT4 inhibitor. STAT4 is a transcription factor that has been shown to be involved in IL-12 responses (see *e.g.*, Thierfelder, W.E. *et al.* (1996) *Nature* 382:171-174; Kaplan, M.H. *et al.* (1996) *Nature* 382:174-177). Accordingly, IL-12 responses in a subject can be inhibited through administration of a STAT4 inhibitor.

Other inhibitors of IL-12 activity that are known in the art also can be used in the methods of the invention. For example, PCT Publication WO 96/40093 discloses biphenyl derivatives for antagonizing IL-12 induced immune responses. Such biphenyl derivatives can be used as IL-12 antagonists in the methods of the invention.

In another embodiment, the method of the invention involves administration of an agent that is an NK cell antagonist. The NK cell antagonist is administered to the subject at a dosage and by a route sufficient to inhibit IFN-γ activity in the subject. Preferably, the NK cell antagonist is an antibody, antibody fragment, or engineered binding protein that specifically binds to NK/NK-like cells such that the cells are depleted or eliminated in a subject. Accordingly, preferred NK cell antagonists bind to specific surface markers present on NK/NK-like cells. Particular preferred NK cell antagonists are anti-asialo-GM1 antibodies and NK1.1. antibodies, which have been shown to be effective in depleting NK/NK-like activity from a subject (see Example 10; Axelsson, L-G. *et al.* (1996) *Inflamm. Res.* 45:181-191; Heremans, H. *et al.* (1994) *Eur. J. Immunol.* 24:1155-1160). Other NK cells antagonists include agents which inhibit factors such as IL-1α, IL-1β and TNF, which induce IFN-γ production from NK cells. Preferably, the agent is an antibody, antibody fragment, or an engineered binding protein. Example of particularly preferred agents are anti-IL-1α antibodies, anti-Iβ antibodies and anti-TNF antibodies (e.g., D2E7). Anti-TNF antibodies are described more fully in U.S. Patent Application Serial No. 09/125,098.

Other antibodies that target surface markers that identify NK/NK-like cells include antibodies reactive with Fc-IgG receptors B73.1 and Leu 11 (CD16) (Lancer, L.L. *et al.* (1983) *J. Immunol.* 131:1789-1796; Perussia, B. *et al.* (1983) *J. Immunol.* 130:2133-2141), Leu 7 (anti-HNK1, which identify 40-60% of NK cells; Abo, T. and Balch, C.M. (1981) *J. Immunol.* 127:1024-1029), and OKT11 (CD2, which identify 50%

or more of NK cells; Lancer, L.L. *et al. supra*; Perussia, B. et al., *supra*). Other NK cell-specific surface antigens, and antibodies thereto, that have been described include the DX1 antigen (see PCT Publication WO 95/02611), the PEN5-alpha and PEN5-beta glycoprotein pair (see PCT Publication WO 95/06247) and the NKB1 antigen (see PCT Publication WO 95/20604).

The exact dosage and regimen for administering an NK cell antagonist will necessarily depend upon the needs of the subject being treated, the type of treatment, the efficacy of the compound and the degree of disease severity in the subject. However, a nonlimiting example of a dosage range for anti-NK/NK-like cell antibodies is from about 0.01 to about 150 mg/kg body weight/day. A single dosage of antibody may be sufficient to deplete or eliminate NK/NK-like cell activity or, alternatively, multiple dosages may be given as needed to deplete or eliminate NK/NK-like cell activity. Preferably, the NK antagonist is administered by an intravenous or intraperitoneal route.

In another embodiment, the method of the invention involves administration of an agent that is an T cell antagonist. The T cell antagonist is administered to the subject at a dosage and by a route sufficient to inhibit IFN-γ activity in the subject. Preferably, the T cell antagonist is an antibody, antibody fragment, or engineered binding protein that specifically binds to T cells such that the cells are depleted in a subject. Accordingly, preferred T cell antagonists bind to specific surface markers present on T cells (e.g., CD4, CD28, CD7, etc.). Particular preferred T cell antagonists are anti-CD4 antibodies, anti-CD28 antibodies, and anti-CD7 antibodies.

The exact dosage and regimen for administering a T-cell antagonist will necessarily depend upon the needs of the subject being treated, the type of treatment, the efficacy of the compound and the degree of disease severity in the subject. However, a nonlimiting example of a dosage range for anti-T cell antibodies is from about 0.01 to about 150 mg/kg body weight/day. A single dosage of antibody may be sufficient to deplete or eliminate T cell activity or, alternatively, multiple dosages may be given as needed to deplete or eliminate T cell activity. Preferably, the T antagonist is administered by an intravenous or intraperitoneal route.

In the methods of the invention, an agent which antagonizes a target that regulates production of interferon-γ (IFN-γ) is administered to a subject in combination with one or more corticosteroids. The term "in combination with" a corticosteroid is intended to include simultaneous administration of the agent and the corticosteroid, administration of the agent first, followed by the corticosteroid and administration of the corticosteroid first, followed by the agent. Any of the therapeutically useful corticosteroids known in the art can be used in the methods of the invention. Corticosteroids are typically classified by the duration of their tissue effects: short acting compounds (*e.g.*, beclomethasone, flunisolide, hydrocortisone, cortisone), intermediate acting compounds (*e.g.*, prednisone, prednisolone, methylprednisolone, triamcinolone, deflazacort) and long-acting compounds (*e.g.*, dexamethasone, beta methasone). One or more corticosteroids can be administered to the subject by a route and at a dosage effective to achieve the desired therapeutic results. Examples of suitable routes of delivery include intravenous administration, intrarectal administration, subcutaneous administration, intramuscular administration, oral administration, topical administration, administration by inhalation (*e.g.*, bronchial administration), and local injection (*e.g.*, intra-joint). The exact dosage and regimen for administering a corticosteroid to the subject will necessarily depend upon the needs of the subject being treated, the type of treatment, the efficacy of the compound and the degree of disease severity in the subject. However, a nonlimiting example of a dosage range for corticosteroids is from about 0.05 mg/day to about 1 gm/day, depending upon the particular corticosteroid used. Certain preferred dosage regimens utilize alternate day administration (*e.g.*, high dose intravenous pulse therapy).

Corticosteroid formulations suitable for administration are well known in the art and commercially available. For example, dexamethasone acetate, 16 mg/ml aqueous suspension, is suitable for intramuscular injection in the treatment of rheumatoid, dermatological, ophthalmic, gastrointestinal, hematologic, neoplastic, allergic conditions and collagen disorders. Nonlimiting examples of dosages include 0.8 mg, 1.6 mg, 4 mg and 16 mg of dexamethasone per injection.

Hydroxycortisone is available as a sterile aqueous solution for intravenous, intramuscular, and subcutaneous injection and is a potent anti-inflammatory agent for conditions such as osteoarthritis, rheumatoid arthritis, juvenile rheumatoid arthritis, acute and chronic bursitis. The preferred initial dosages can be from 15 mg to 250 mg per human subject per day. Preferred dosages are oral or parenteral, and can be administered in half the daily dosage, administered twice per day, or other multiples. Hydrocortisone injection can be added to sodium chloride injection or dextrose injection and administered by intravenous drip. Hydrocortisone valerate, 0.2% by weight, is formulated as a cream for topical use under the name Westcort. Preferred dosages comprise application to affected areas several times daily as thin films.

Beconase (beclomethasone) is available for inflammation of the nasal passages and sinuses, for example, as 8.4 mg for 200 metered spray doses in a 0.042% aqueous suspension, delivered in metered doses of 100 mg containing 42 μg per metered dose, such that daily nasal delivery consists of preferably 42 μg per nostril, 84 μg per nostril, 168 μg per nostril, 336 μg per nostril, 672 μg per nostril, or 1,344 μg per nostril. It is preferably delivered, for example, in an aqueous medium in suspension with microcrystalline cellulose, carboxymethylcellulose sodium, dextrose, benzalkonium chloride, polysorbate 80, and 0.25% v/w phenylethyl alcohol. Additional propellants and media are included in some formulations.

In certain embodiments in which an agent of the invention is coadministered with a corticosteroid, the agent is administered systemically to regulate IFN-γ production systemically while the corticosteroid is administered either locally or systemically. For example, in certain embodiments when a phosphodiesterase IV inhibitor or a beta-2 agonist is administered together with a corticosteroid, the phosphodiesterase IV inhibitor or beta-2 agonist is administered systemically, such as intravenously or orally, and the corticosteroid is administered either systemically or locally. Additionally, in certain embodiments of the methods of the invention, use of a phosphodiesterase IV inhibitor or a beta-2 agonist in combination with a corticosteroid for the treatment of asthma is specifically excluded from the scope of the invention.

Furthermore, the methods of the invention also include administering the agent which antagonizes a target that regulates production of interferon-γ (IFN-γ) in combination with a corticosteroid. The agent and the corticosteroid can be administered together in the same pharmaceutically acceptable carrier or the agent and the corticosteroid are administered separately in independently chosen pharmaceutically acceptable carriers. In yet another embodiment, the agent is administered in a pharmaceutically acceptable carrier and the corticosteroid is administered with or without a pharmaceutically acceptable carrier. In yet another embodiment, the corticosteroid is administered in a pharmaceutically acceptable carrier and the agent is administered with or without a pharmaceutically acceptable carrier.

The methods of the invention can be used in the treatment of a variety of inflammatory and immunological disorders. For example, in a preferred embodiment, the subject to be treated is suffering from septic shock (*i.e.*, the methods of the invention allow for corticosteroids to be used in the treatment of septic shock). In another preferred embodiment, the subject to be treated is suffering from Crohn's disease. In yet another preferred embodiment, the subject to be treated is suffering from asthma. In still another preferred embodiment, the subject to be treated is suffering from graft-versus-host disease or transplant rejection. In still another preferred embodiment, the subject to be treated is suffering from an autoimmune disease. In yet another embodiment, the subject is suffering from complications associated with post-surgical stress, Still's disease, or leukemia.

In another embodiment, the subject to be treated is suffering from an immunoinflammatory disease or disorder. Non-limiting examples of immunoinflammatory diseases and disorders that may be treated according to the invention include asthma, adult respiratory distress syndrome, systemic lupus erythematosus, inflammatory bowel disease (including Crohn's disease and ulcerative colitis), multiple sclerosis, insulin-dependent diabetes mellitus, autoimmune arthritis (including rheumatoid arthritis, juvenile rheumatoid arthritis, psoriatic arthritis), inflammatory pulmonary syndrome, pemphigus vulgaris, idiopathic thrombocytopenic purpura, autoimmune meningitis, myasthenia gravis, autoimmune thyroiditis, dermatitis (including atopic dermatitis and eczematous dermatitis), psoriasis, Sjögren's Syndrome

(including keratoconjunctivitis sicca secondary to Sjögren's Syndrome), alopecia areata, allergic responses due to arthropod bite reactions, aphthous ulcer, iritis, conjunctivitis, keratoconjunctivitis, cutaneous lupus erythematosus, scleroderma, vaginitis, proctitis, drug eruptions (such as Stevens-Johnson syndrome), leprosy reversal reactions, erythema nodosum leprosum, autoimmune uveitis, allergic encephalomyelitis, aplastic anemia, pure red cell anemia, idiopathic thrombocytopenia, polychondritis, Wegener's granulomatosis, chronic active hepatitis, Graves ophthalmopathy, primary biliary cirrhosis, uveitis posterior and interstitial lung fibrosis.

In another embodiment, the subject to be treated is suffering from an acute inflammatory disorder. Examples of acute inflammatory disorders including graft versus host disease, transplant rejection, septic shock, endotoxemia, Lyme arthritis, infectious meningitis (*e.g.*, viral, bacterial, Lyme disease-associated), an acute episode of asthma and acute episodes of an autoimmune disease.

In yet another embodiment, the subject to be treated is suffering from a chronic inflammatory disorder. Nonlimiting examples of chronic inflammatory disorder which can be treated include asthma, rubella arthritis, and chronic autoimmune diseases, such as systemic lupus erythematosus, psoriasis, inflammatory bowel disease, including Crohn's disease and ulcerative colitis, multiple sclerosis and rheumatoid arthritis.

In certain cases, agents that antagonize a particular target that regulates IFN-γ in the subject may be preferred for the treatment of a particular disorder. For example, although not intending to be limited by mechanism, disorders in which IFN-γ is preferentially or predominantly produced by NK cells preferably are treated using an agent that antagonizes IL-18 (such as an ICE inhibitor) or directly antagonizes the NK cells (i.e., an NK cell antagonist, such as an anti-NK/NK-like cell antibody), in combination with a corticosteroid. Alternatively, disorders in which IFN-γ is preferentially or predominantly produced by T cells preferably are treated using an agent that antagonizes IL-12 (*e.g.*, an anti-IL-12 antibody or an agent that stimulates intracellular production of cAMP), in combination with a corticosteroid. In other circumstances, it may be beneficial to use both an IL-18 antagonist and an IL-12

antagonist (*e.g.*, in the treatment of disorders in which IFN-γ production is contributed by both T cells and NK cells).

The agent and the corticosteroid are administered to the subject in need of treatment according to standard routes of drug delivery well known in the art, the particular route and dosage of the agent and the corticosteroid being selected depending upon the needs of the subject being treated, the type of treatment, the efficacy of the compound and the degree of disease severity in the subject. The agent and the corticosteroid are administered at an "effective therapeutic dose", which means that amount of the therapeutic composition which, when administered to a subject produces an amelioration of a disorder in comparison to those subjects which have not been administered the drug. One of ordinary skill in the art can determine and prescribe the effective amount of the therapeutic agents and corticosteroid required. The agents and corticosteroids of the invention are administered to subjects in biologically compatible forms suitable for pharmaceutical administration *in vivo* to produce a desired therapeutic response. By "biologically compatible form suitable for administration *in vivo*" is meant a form of the drug to be administered in which any toxic effects and side effects are outweighed by the therapeutic effects of the composition. Moreover, an agent of the invention that antagonizes a target that regulates production of IFN-γ in a subject is administered to the subject at a dosage and by a route sufficient to inhibit IFN-γ production in the subject. Similarly, an IL-12 antagonist or IL-18 antagonist of the invention is administered to a subject at a dosage and by a route sufficient to inhibit IL-12 activity or IL-18 activity, respectively, in the subject.

Animal models of inflammatory and immunological disorders that are accepted in the art as being models of human disease can be used to evaluate various therapeutic regiments of the invention. For example, the *P.acnes*/LPS model of septic shock described in the Examples can be used to evaluate the efficacy of therapeutic regimens for the treatment of septic shock. Numerous animal models of autoimmune disease are known in the art and can be applied to the methods herein to evaluate the efficacy of therapeutic regimens, nonlimiting examples of which include experimental colitis (see *e.g.*, Neurath, M.F. *et al.* (1995) *J. Exp. Med.* 182:1281-1290), experimental allergic

encephalomyelitis (see *e.g.*, Leonard, J.P. *et al.* (1995) *J. Exp. Med.* 181:381-386), collagen-induced arthritis (Banerjee, S. *et al.* (1989) *J. Immunol.* 142:2237-2243) and the human TNFα transgenic model of polyarthritis (see *e.g.*, Keffer, J. *et al. EMBO J* (1991) 10:4025-4031). For therapeutic regimens involving inhibition of ICE activity, ICE deficient mice can be used as a model of complete inhibition of ICE activity. Such ICE -/- mice have been described in the art (see *e.g.*, Li, P., *et al.* (1995) *Cell* 80:401-411 and PCT Publication No. WO 96/12025).

The methods of the invention are useful for modulating corticosteroid responsiveness in a variety of clinical settings. For example, in one embodiment, the methods of the invention are used to reverse steroid resistance in a subject, as compared to when a corticosteroid alone is administered to the subject. In another embodiment, the methods of the invention are used to increase steroid sensitivity in a subject, as compared to when a corticosteroid alone is administered to the subject. In yet another embodiment, the corticosteroid is administered to a subject according to a schedule that reduces the dosage of the corticosteroid over time and the method ameliorates a steroid rebound effect associated with administration of reduced dosages of the corticosteroid. In a further advantageous embodiment, the methods of the invention modulate corticosteroid activity, such that the corticosteroids can be tapered, e.g., tapered to zero. The ability of the methods of the invention to increase steroid sensitivity (*i.e.*, to have a "steroid sparing effect") may therefore allow for the use of corticosteroid therapy in clinical situations in which such therapy previously has been contraindicated. For example, use of the methods of the invention may allow for corticosteroid therapy in patients that previously could not be treated because of detrimental side effects of corticosteroid therapy, such as young children (*e.g.*, in juvenile rheumatoid arthritis), patients with uncontrolled diabetes and patients with hypertension.

Another aspect of the invention pertains to a method for modulating responsiveness to a corticosteroid in a subject, comprising:

selecting a subject in need of modulation of responsiveness to a corticosteroid; and

administering to the subject an agent which antagonizes a target that regulates production of interferon-γ (IFN-γ) in the subject, the agent being administered at a dosage and by a route sufficient to inhibit production of IFN-γ in the subject, such that responsiveness of the subject to a corticosteroid is modulated as compared to when a corticosteroid alone is administered to the subject.

The subject that is selected for treatment according to the method can be, for example a subject that is resistant to a corticosteroid prior to administration of the agent. Alternatively, the subject that is selected for treatment can be a subject that is responsive to a corticosteroid prior to administration of the agent but that exhibits increased sensitivity to the corticosteroid after administration of the agent. One examples of such a subject is a patients suffering from a steroid dependent disorder, which disorder can be treated with lower doses of corticosteroids when treated in accordance with the methods of the invention. Another example of such a subject is a patient for whom steroid therapy has been contraindicated due to side effects when the corticosteroid is administered alone but who can tolerate a lower dosage of corticosteroid when the corticosteroid is administered in accordance with the methods of the invention . Still further, the subject that is selected for treatment according to the method can be a subject undergoing corticosteroid therapy but in whom corticosteroid therapy is to be stopped, such that administration of the agent ameliorates a steroid rebound effect in the subject. Agents for antagonizing a target that regulates production of IFN-γ in the subject are as described hereinbefore.

In another embodiment, the invention pertains to methods for modulating responsiveness to a corticosteroid in a subject, by administering an agent which antagonizes a target that regulates production of interferon-γ (IFN-γ) in the subject in combination with a corticosteroid, such that responsiveness of the subject to the corticosteroid is modulated. For example, the agent may be an IL-18 antagonist or an IL-12 antagonist.

In yet another embodiment, the invention features a method for regulating the production of IFN-γ in a subject, by administering to a subject an agent which antagonizes a target that regulates production of IFN-γ in the subject in combination

with a corticosteroid. For example, the agent may be an IL-18 antagonist or an IL-12 antagonist.

II. Pharmaceutical Compositions

Another aspect of the invention pertains to pharmaceutical compositions for modulating responsiveness to corticosteroids. In one embodiment, the pharmaceutical composition of the invention comprises an agent which antagonizes a target that regulates production of interferon-γ (IFN-γ) in the subject, a corticosteroid and a pharmaceutically acceptable carrier. As discussed above, the target that is antagonized can be, for example, IL-18, IL-12, or NK cells (*i.e.*, the pharmaceutical composition can comprise an IL-18 antagonist, an IL-12 antagonist or an NK cell antagonist, as described hereinbefore, a corticosteroid and a pharmaceutically acceptable carrier). A particularly preferred IL-18 antagonist is an anti-IL-18 monoclonal antibody.

In a preferred embodiment, a pharmaceutical composition of the invention comprises an inhibitor of a caspase family protease, a corticosteroid and a pharmaceutically acceptable carrier. Examples of inhibitors of caspase family proteases, and nonlimiting exemplary dosages, are described hereinbefore. In a preferred embodiment, the inhibitor of the caspase family protease is an ICE inhibitor.

In yet another embodiment, a pharmaceutical composition of the invention comprises an IL-12 antagonist, a corticosteroid and a pharmaceutically acceptable carrier. Examples of such IL-12 antagonists are described hereinbefore. In a preferred embodiment, the IL-12 antagonist is an anti-IL-12 monoclonal antibody. In another preferred embodiment, the IL-12 antagonist is a phosphodiesterase IV inhibitor. In yet another preferred embodiment, the IL-12 antagonist is a beta-2 agonist. In another preferred embodiment the IL-12 antagonist is J695.

In still another embodiment, a pharmaceutical composition of the invention comprises an NK cell antagonist, a corticosteroid and a pharmaceutically acceptable carrier. Examples of such NK cell antagonists are described hereinbefore. In a preferred embodiment, the anti-NK cell antagonist is an anti-NK/NK-like cell antibody, preferably an anti-asialo-GM1 antibody or an NK1.1 antibody. Other examples of NK cell

antagonists include anti-IL-1α antibodies, anti-IL-1β antibodies, and anti-TNF antibodies (e.g., D2E7).

As used herein the term "pharmaceutically acceptable carrier" is intended to include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active compound, use thereof in the compositions is contemplated. Supplementary active compounds can also be incorporated into the compositions.

A pharmaceutical composition of the invention is formulated to be compatible with its intended route of administration. For example, solutions or suspensions used for parenteral, intradermal, or subcutaneous application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor EL™ (BASF, Parsippany, NJ) or phosphate buffered saline (PBS). In all cases, the composition must be sterile and should be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for

example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyetheylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions can be prepared by incorporating the active compound in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle which contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying which yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

Oral compositions generally include an inert diluent or an edible carrier. They can be enclosed in gelatin capsules or compressed into tablets. For the purpose of oral therapeutic administration, the active compound can be incorporated with excipients and used in the form of tablets, troches, or capsules. Oral compositions can also be prepared using a fluid carrier for use as a mouthwash, wherein the compound in the fluid carrier is applied orally and swished and expectorated or swallowed. Pharmaceutically compatible binding agents, and/or adjuvant materials can be included as part of the composition. The tablets, pills, capsules, troches and the like can contain any of the following ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a disintegrating agent such as alginic acid, Primogel, or corn starch; a lubricant

such as magnesium stearate or Sterotes; a glidant such as colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; or a flavoring agent such as peppermint, methyl salicylate, or orange flavoring.

In one embodiment, the active compounds are prepared with carriers that will protect the compound against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art. The materials can also be obtained commercially from Alza Corporation and Nova Pharmaceuticals, Inc. Liposomal suspensions (including liposomes targeted to infected cells with monoclonal antibodies to viral antigens) can also be used as pharmaceutically acceptable carriers. These may be prepared according to methods known to those skilled in the art, for example, as described in U.S. Patent No. 4,522,811.

The pharmaceutical compositions of the invention can be formulated for administration by a particular route of administration, such as oral administration, intravenous administration, ophthalmic administration, and the like.

In a preferred embodiment, a pharmaceutical composition of the invention is formulated for topical administration. Accordingly, an agent which antagonizes a target that regulates production of interferon-γ (IFN-γ) in the subject, a corticosteroid and a pharmaceutically acceptable carrier can be formulated into a cream, salve, ointment and the like suitable for application to the skin.

In another preferred embodiment, a pharmaceutical composition of the invention is formulated for administration by inhalation. Accordingly, an agent which antagonizes a target that regulates production of interferon-γ (IFN-γ) in the subject, a corticosteroid and a pharmaceutically acceptable carrier can be formulated into a nasal spray or an inhalant to allow for delivery of the therapeutic agents to the nasal or sinus passages or the lungs (*e.g.*, the bronchial passages) by inhalation.

The invention also provides a packaged agent for use in combination therapy with corticosteroids, comprising an agent which antagonizes a target that regulates production of IFN-γ in the subject and directions instructing the administration of the agent in combination with corticosteroids using the methods of the invention. In a further embodiment, the composition also comprises a pharmaceutically effective carrier. Examples of preferred agents include ICE inhibitors, anti-IL-12 antibodies, and anti-IL-18 antibodies.

This invention is further illustrated by the following examples which should not be construed as limiting. The contents of all references and published patents and patent applications cited throughout the application are hereby incorporated by reference.

EXEMPLIFICATION OF THE INVENTION

EXAMPLE 1: Inhibition of ICE Activity in a Septic Shock Model Results in Steroid Responsiveness

In this example, the effect of inhibiting ICE activity on steroid responsiveness in septic shock was examined. A model of septic shock was induced in ICE-deficient (ICE -/-) and wild type (ICE +/+) mice, followed by treatment with a corticosteroid. The ICE -/- mice serve as a model of complete inhibition of ICE activity (see Li, P., *et al.* (1995) *Cell* 80:401-411 for further description of the ICE deficient mice). The responsiveness of the animals to corticosteroid treatment was determined by monitoring the levels of the inflammatory cytokine TNFα in the sera of the mice.

ICE-deficient and wild type mice first were sensitized with *Propionibacterium acnes* cell wall material (1 mg per mouse) to induce low grade inflammation and six days later were challenged with lipopolysaccharide (LPS) (1 μg per mouse in 0.1 ml of saline i.v.). Thirty minutes after LPS administration, the mice were treated with the corticosteroid dexamethasone (4 mg/kg per mouse in 0.5 ml 95% saline/0.5% ethanol, i.p.). Control mice were treated with vehicle alone. All mice were bled 90 minutes after

LPS administration and the serum samples were analyzed for the presence of TNFα by standard ELISA.

The results are shown in Figure 1. Wild type and ICE deficient mice treated with vehicle alone had similar levels of serum TNFα. Treatment of wild type mice with dexamethasone did not significantly affect serum TNFα levels, demonstrating their resistance to steroid treatment in this septic shock model. In contrast, treatment of the ICE deficient mice with dexamethasone suppressed serum TNFα levels by 74% ($p<0.002$). These data indicate that inhibition of ICE activity reverses resistance to steroid treatment in a septic shock model.

EXAMPLE 2: Inhibition of ICE Activity in a Septic Shock Model Increases Steroid Sensitivity

In this example, the effect of inhibiting ICE activity on steroid sensitivity in septic shock was examined. The same LPS/*P. acnes* model of septic shock described in Example 1 was used, except that ICE deficient and wild type mice were pretreated with vehicle or a corticosteroid 15 minutes prior to challenge with LPS. The responsiveness of the animals to corticosteroid treatment again was determined by monitoring the levels of the inflammatory cytokine TNFα in the sera of the mice.

ICE-deficient and wild type mice first were sensitized with *Propionibacterium acnes* cell wall material (1 mg per mouse) to induce low grade inflammation and six days later were challenged with lipopolysaccharide (LPS) (1 μg per mouse in 0.1 ml of saline i.v.). Fifteen minutes prior to LPS challenge, the animals were treated with decreasing amounts of the corticosteroid dexamethasone (0.05, 0.005 or 0.0005 mg/kg per mouse in 0.5 ml 95% saline/0.5% ethanol, i.p.). Control mice were treated with vehicle alone. All mice were bled 90 minutes after LPS administration and the serum samples were analyzed for the presence of TNFα by standard ELISA.

The results are shown in Figure 2. Both the wild type and the ICE deficient mice exhibited responsiveness to pretreatment with 0.05 mg/kg of dexamethasone. In contrast, ICE deficient mice pretreated with only 0.005 or 0.0005 mg/kg dexamethasone

exhibited 76% and 78% ($p<0.005$) lower serum TNFα levels, respectively, compared to the lack of TNFα suppression in the wild type animals similarly treated. These data indicate that inhibition of ICE activity results in increased steroid sensitivity in a septic shock model, since 10-100 fold lower doses of dexamethasone were therapeutically effective in the ICE deficient animals as compared to the wild type animals.

EXAMPLE 3: A Phosphodiesterase IV Inhibitor Reduces IL-12 Production

In this example, the effect of a phosphodiesterase IV inhibitor, Rolipram, on LPS-induced IL-12 production was examined. B6 mice were pretreated with vehicle or Rolipram (30 mg/kg in 0.5 ml 0.1% methyl cellulose, i.p.) 15 minutes prior to challenge with LPS (10 μg/mouse, i.v.). Ninety minutes following LPS administration, the mice were bled and serum levels of IL-12 were determined by standard ELISA.

The results are shown in Figure 3. Mice pretreated with Rolipram had 70% lower serum IL-12 levels than mice pretreated with vehicle alone. These data indicate that phosphodiesterase IV inhibitors are effective for inhibiting the production of LPS-induced IL-12.

EXAMPLE 4: Cleavage of IL-18 by Caspase Family Proteases

The ability of various recombinant (*i.e.*, E. coli expressed) caspase family proteases to cleave precursor IL-18 (proIL-18) to mature IL-18 was tested in an *in vitro* proteolysis assay. Cleavage of poly(ADP-ribose) polymerase (PARP) was used as a positive control. The results are summarized below in Table 1.

Table 1 Proteolysis of proIL-18 by Recombinant Caspases

Caspase*		Concentration (μg/ml)	% Cleavage proIL-18	% Cleavage PARP
ICE	(1)	1.25	100	99
ICH-2	(4)	5.00	82	93
$ICE_{rel}III$	(5)	20.00	55	90
CPP32	(3)	5.00	0[a]	100
Mch2	(6)	10.00	2	96
Mch3	(7)	5.00	32[b]	97
ICH-1	(2)	75.00	5	98

* Caspases are numbered in parenthesis as recommended in Alnemri *et al.* (1996) *Cell* 87:171.

[a] CPP32 (5 μg/ml) cleaved proIL-18 but generated a 12 kDa and a 10 kDa fragment instead of the expected 18 kDa fragment.

[b] Unlike other caspases, the Mch3 precursor expressed in *E. coli* does not undergo autocatalysis to generate an active protease. Addition of ICE was required to initiate Mch3 autocatalysis and generate an active Mch3 protease. Partial cleavage of proIL-18 by Mch3 is mediated by the presence of ICE in the Mch3 preparation.

EXAMPLE 5: Treatment of Septic Shock

Patients who present in a clinical setting with septic shock (*e.g.*, in conjunction with infected abrasions, projectile wounds, or systemic bacteremias from other sources) are administered agent selected from an ICE inhibitor, a phosphodiesterase IV inhibitor (*e.g.*, rolipram, 30 mg/kg) and an anti-IL-12 monoclonal antibody, together with a corticosteroid (*e.g.*, high dose methylprednisolone, 1 gm/day, i.v.). The corticosteroid and the agent can be administered simultaneously, or alternatively, the agent can be administered before or after corticosteroid administration. Patients are also treated with appropriate antibiotic therapy.

EXAMPLE 6: **Treatment of Transplant Rejection**

Patients who are to receive a kidney transplant are administered an agent selected from ICE inhibitor, a phosphodiesterase IV inhibitor (*e.g.*, rolipram, 30 mg/kg) and an anti-IL-12 monoclonal antibody together with a corticosteroid (*e.g.*, oral prednisone, 25-75 mg/day). Treatment preferably is begun prior to receipt of the donated kidney (*e.g.*, drug administration may begin 24 hours prior to receipt of the donated kidney), with dosages to be repeated as needed (*e.g.*, every 12 hours). The corticosteroid and the agent can be administered simultaneously, or alternatively, the agent can be administered before or after corticosteroid administration. Patients are also treated with additional immunosuppressive therapy (such as cyclosporin A treatment or OKT3 antibody treatment) so that immune rejection and inflammatory response are simultaneously suppressed.

EXAMPLE 7: **Amelioration of the Steroid Rebound Effect**

Patients with asthma, allergic rhinitis inflammation or rheumatoid arthritis who are undergoing treatment with a corticosteroid inhalant or with systemic corticosteroids, and who are to enter a scheduled withdrawal from steroid treatment, are administered an agent selected from an ICE inhibitor, a phosphodiesterase IV inhibitor (*e.g.*, rolipram, 30 mg/kg) and an anti-IL-12 monoclonal antibody. Patients are preferably treated prior to the tapering or discontinuance of steroid treatment to ameliorate the steroid rebound effect that can result from cessation of steroid therapy. As needed, patients can be treated with additional nonsteroidal anti-inflammatory agents.

EXAMPLE 8: **Treatment of an Acute Episode of an Autoimmune Disease**

Patients suffering from an acute flare-up of an autoimmune disease such as inflammatory bowel disease (*e.g.*, ulcerative colitis or Crohn's disease) are administered an agent selected from ICE inhibitor, a phosphodiesterase IV inhibitor (*e.g.*, rolipram, 30

prednisone, 25-75 mg/day). The corticosteroid and the agent can be administered simultaneously, or alternatively, the agent can be administered before or after corticosteroid administration. Patients can also be treated with additional immunosuppressive therapy to control the acute flare up of the autoimmune disease.

EXAMPLE 9: Treatment of a Chronic Autoimmune Disease

Patients suffering from chronic autoimmune disease such as Crohn's disease are administered an agent selected from ICE inhibitor, a phosphodiesterase IV inhibitor (*e.g.*, rolipram, 30 mg/kg) and an anti-IL-12 monoclonal antibody together with a corticosteroid (*e.g.*, oral prednisone, 25-75 mg/day). The corticosteroid and the agent can be administered simultaneously, or alternatively, the agent can be administered before or after corticosteroid administration. Patients can also be treated with additional immunosuppressive therapy to control the autoimmune disease.

EXAMPLE 10: Inhibition of IFN-γ Production by Elimination of NK Cells

In this example, shock was induced in mice by high dose LPS treatment (40 mg/kg LPS administered intravenously). The effect of depleting NK cells on the production of various cytokines in the mice and on mortality was examined by administering an anti-asialo-GM1 antibody (anti-ASGM1) intravenously 10 minutes prior to LPS administration. Control animals received Rabbit IgG. The effect of ASGM1 treatment on production of IL-1β, TNFα and IFN-γ, as well as on mortality, is summarized below in Table 2:

Table 2

Treatment	# Mice	Cytokine Production (pg/ml)			Mortality (30 Hrs)
		IL-1β	TNFα	IFN-γ	% Survival
LPS + Rabbit IgG (control)	6	1946 ± 483	5857 ± 1071	1663 ± 811	0 (n=10)*
LPS + ASGM1 Antibodies	6	1647 ± 482	5453 ± 1103	363 ± 108	90 (n=10)

* All animals died within 15 hrs.

These results indicate that elimination of NK cells by anti-asialo-GM1 antibody treatment reduces IFN-γ production and prolongs survival after LPS administration in high-dose LPS shock.

EXAMPLE 11: Effect of an ICE Inhibitor and Corticosteroid in Septic Shock Model

In this example, the LPS/*P. acnes* model of septic shock described in Examples 1 and 2 was used to examine the effect of an ICE inhibitor in combination with a corticosteroid. B6 mice were implanted with a 24 hour osmotic pump containing the ICE inhibitor acetyl-tyrosine-valine-alanine-aspartic acid-CHO (Ac-YVAD-CHO) (100 mg/kg), or a vehicle control, subcutaneously 18 hours before LPS injection. LPS was injected intravenously (0.01 μg/mouse or 10 μg/mouse) at time zero. All mice were injected with 5 mg/kg of dexamethasone intraperitoneally 30 minutes after LPS injection. The responsiveness of the animals to corticosteroid treatment was determined by monitoring the levels of the inflammatory cytokine TNFα, as well as interleukin-6 (IL-6) and interleukin-1β (IL-1β), in the sera of the mice. All mice were bled 90 minutes after LPS administration and the serum samples were analyzed for the presence of TNFα, IL-6 and IL-1β by standard methods.

The results are shown in Figures 4, 5 and 6, for TNFα, IL-6 and IL-1β, respectively. The data demonstrate that mice treated with both the ICE inhibitor Ac-YVAD-CHO and dexamethasone had significantly lower serum levels of TNFα, IL-6 and IL-1β, when either 0.01 μg or 10 μg of LPS was used to induce septic shock. As previously shown in Example 1, treatment of mice with dexamethasone alone did not significantly affect serum TNFα levels, demonstrating the resistance of the mice to steroid treatment alone in this septic shock model. In contrast, treatment of the mice with both dexamethasone and the ICE inhibitor suppressed serum TNFα levels by 96% ($p<0.005$), in mice treated with 0.01 μg of LPS, and by 86% ($p<0.005$), in mice treated with 10 μg of LPS. Moreover, serum IL-6 levels were reduced 95% ($p<0.00005$) and 91% ($p<0.00005$), respectively, and serum IL-1β levels were reduced 94% ($p<0.001$) and 92% ($p<0.0002$), respectively. These data indicate that inhibition of ICE activity using an ICE inhibitor reverses resistance to steroid treatment in a septic shock model.

EXAMPLE 12: Synthesis of Hydroxamate ICE Inhibitors

3-Benzyloxycarbonylamino-4-oxo-5-phenylacetylaminooxy-pentanoic acid

Step A N-(Phenylmethoxy)-benzeneacetamide [(0.760 g, 3.15 mmol), prepared by the method of Hearn M.T.W. and Ward A.D. (Aust. J. Chem., 1969;22:1731)] was taken up in 10 mL of CH_3CN and treated with dimethylamino-pyridine (DMAP) (50 mg) and di-tert-butyl dicarbonate (0.824 g, 3.78 mmol). The reaction was allowed to stir under Argon for 12 hours, then diluted with ethyl acetate (EtOAc) and washed with 3M $K_2S_2O_5$ (1 x 10 mL), $NaHCO_3$ (1 x 10 mL). The organic layer was dried over Na_2SO_4, filtered, and concentrated. Purification by chromatography (SiO_2, 9:1 hexane-EtOAc) afforded 0.910 g (84%) of 1,1-dimethylethyl(phenylacetyl)phenyl-methoxy)carbamate as a clear, viscous oil.

^1H NMR (400 MHz, DMSO-d_6): ä 7.41 [m, 5H], 7.32 [m, 2H], 7.24 [m, 3H], 4.90 [s, 2H], 4.09 [s, 2H], 1.48 [s, 9H]. · IR (thin film) 3063, 3032, 2979, 2935, 2886, 1777, 1736, 1497, 1455, 1370, 1302 cm^{-1}. Mass Spectra (MS) (Chemical Ionization [CI] - NH_3) 342 (M^++H).

Elemental Analysis:

Calculated for $C_{20}H_{23}NO_4 \cdot 0.051\ CH_2Cl_2$: C, 69.66; H, 6.74; N, 4.05.

Found: C, 69.66; H, 6.83; N, 3.99.

Step B 1,1-Dimethylethyl (phenylacetyl)(phenyl-methoxy)carbamate (810 mg, 2.37 mmol) was dissolved in 75 mL of dry THF and 90 mg of 5% $Pd/BaSO_4$ was added. The reaction was treated with H_2 (20 psi) for 20 hours. The reaction was filtered through Celite and concentrated to obtain 588 mg (99%) of 1,1-dimethyl-ethyl hydroxy-(phenylacetyl)carbamate as an oil. No further purification was done.

^{1}H NMR (400 MHz, $CDCl_3$): ä 8.22 [s, 1H], 7.31 [m, 5H], 4.24 [s, 2H], 1.55 [s, 9H].

Step C (S)-5-Bromo-4-oxo-3-[[(phenylmethoxy)-carbonyl]amino]-pentanoic acid, 1,1-dimethylethyl ester [(297 mg, 0.742 mmol), prepared according to the procedure of Dolle R.E., et al., (J. Med. Chem., 1994;37:563-4)], 1,1-dimethylethyl hydroxy(phenyl-acetyl)carbamate (187 mg, 0.742 mmol) and KF (104 mg, 1.85 mmol) were combined in 5 mL dimethylformamide (DMF) and allowed to stir under Ar for 12 hours. The reaction was diluted with EtOAc (15 mL) and washed with water (3 x 15 mL) and brine (1 x 15 mL). The organic layer was dried over Na_2SO_3 and concentrated. Purification by chromatography (SiO_2, 4:1 hexane-EtOAc) yielded 168 mg (40%) of [[[(1,1-dimethylethoxy)-carbonyl](phenylacetyl)amino]oxy]-4-oxo-3-[[((phenylmethoxy)carbonyl]amino]-pentanoic acid, 1,1-dimethylethyl ester as a clear oil.

^{1}H NMR (300 MHz, $CDCl_3$): ä 7.35 [m, 5H], 5.84 [d, J = 9.0 Hz, 1H], 4.79, [A of AB, J = 15.3 Hz, 1H], 4.70 [m, 1H], 4.57 [B of AB, J = 15.3 Hz, 1H], 4.10 [s, 2H], 3.09 [dd, J = 16.8, 4.6 Hz, 1H], 2.79 [dd, J = 16.8, 4.9 Hz, 1H], 1.52 [s, 9H], 1.39 [s, 9H].

IR (thin film) 3374, 2980, 2935, 1726 (br), 1499, 1370, 1298, 1150 cm^{-1}. MS (APCI, Methanol(MeOH)) 571.5 (M^++H).

Elemental Analysis:

Calculated for $C_{30}H_{38}N_2O_9$: C, 63.15; H, 6.71; N, 4.91.

Found: C, 62.76; H, 6.70; N, 4.69.

Step D 3-Benzyloxycarbonylamino-4-oxo-5-phenylacetylaminooxy-pentanoic acid, 1,1 dimethylethyl ester (208 mg, 0.365 mmol) was taken up in 3 mL of 1:1 trifluoroacetic acid (TFA)/CH_2Cl_2 and allowed to stir for 2 hours. Reaction was diluted with acetonitrile (MeCN) (10 mL) and concentrated. The residue was stripped down from MeCN five times. Purification by chromatography (SiO_2, 90:9:1 CH_2Cl_2-acetone-formic acid) afforded 3-benzyloxycarbonylamino-4-oxo-5-phenylacetylaminooxy-pentanoic acid (51 mg, 34%) as a white foam.

^{1}H NMR (300 MHz, $CDCl_3$): ä 8.63 [s, 1H], 7.34 [broad (br) s, 10H], 5.48 [br d, J = 4 Hz, 1H], 5.08 [br dd, J = 16, 12 Hz, 2H], 4.23 [m, 1H], 3.97 [m, 2H], 3.58 [br s, 2H], 2.80 [m, 1H], 2.64 [m, 1H].

IR (KBr) 3305 (br), 2928, 1791, 1772, 1717, 1699, 1685, 1674, 1654, 1521, 1455 cm^{-1}.

MS (APCI, MeOH) 415 (M^++H).

Elemental Analysis:

Calculated for $C_{21}H_{22}N_2O_7 \cdot 0.106\ CF_3CO_2H$: C, 59.73; H, 5.22; N, 6.57.

Found: C, 59.73; H, 5.46; N, 6.28.

The following were prepared from (S)-5-bromo-4-oxo-3-[[(phenylmethoxy)carbonyl]amino]-pentanoic acid, 1,1-dimethylethyl ester in the manner described above, Step C, and Step D.

3-Benzyloxycarbonylamino-4-oxo-5-(2-oxo-pyrrolidin-1-yloxy)-pentanoic acid

Step A Prepared from 1-hydroxy-2-pyrrolidinone [Biswas A. and Miller M.J. (Heterocycles, 1987;26:2849)] in the manner describe above, Step C to give 3-benzyloxycarbonylamino-4-oxo-5-(2-oxo-pyrrolidin-1-yloxy)-pentanoic acid, 1,1-dimethylethyl ester (74%).

^{1}H NMR (400 MHz, $CDCl_3$): ä 7.37 [m, 5H]; 5.88 [br d, J = 8.9 Hz]; 5.16 [A of AB, J = 12.2 Hz, 1H]; 5.11 [B of AB, J = 12.2 Hz, 1H]; 4.95 [A of AB, J = 17.1 Hz, 1H], 4.81 [B of AB, J = 17.1 Hz, 1H], 4.60 [m, 1H], 3.62 [m, 2H], 3.01 [dd, J = 17.1, 4.6 Hz, 1H], 2.75 [dd, J = 17.1, 4.8 Hz, 1H], 2.30 [t, J = 7.95 Hz, 2H], 1.99 [quint, J = 7.5 Hz, 2H], 1.41 [s, 9H]. IR (KBr) 3328 (br), 2976, 2932, 1717, 1701, 1522, 1256 cm^{-1}. MS (APCI, MeOH) 421 (M^++H).

Elemental Analysis:

Calculated for $C_{21}H_{28}N_2O_7 \cdot 0.096$ DMF: C, 59.81; H, 6.76; N, 6.87.

Found: C, 59.56; H, 7.00; N, 6.52.

Step B: Prepared from 3-benzyloxycarbonylamino-4-oxo-5-(2-oxo-pyrrolidin-1-yloxy)-pentanoic acid, 1,1-dimethylethyl ester in the manner described above, Step D to afford 3-benzyloxycarbonylamino-4-oxo-5-(2-oxo-pyrrolidin-1-yloxy)-pentanoic acid (72%). ^{1}H NMR (400 MHz, $CDCl_3$): ä 8.55 [br s, 1H], 7.36 [m, 5H], 5.46 [br d, J = 9.4 Hz, 1H], 5.14 [A of AB, J = 5.2 Hz, 1H], 5.11 [B of AB, J = 5.2 Hz, 1H], 4.23 [m, 1H], 4.19 [A of AB, J = 13.3 HZ, 1H], 3.96 [B of AB, J = 13.3 Hz, 1H], 3.67 [m, 1H], 3.52 [dd, J = 15.1, 7.9 Hz, 1H], 2.84 [dd, J = 16.9, 8.2 Hz, 1H], 2.61 [dd, J = 16.9, 10.9 HZ, 1H], 2.42 [m, 2H], 2.11 [m, 2H]. IR (KBr) 3408 (br), 2926, 1791, 1717, 1700, 1540, 1268, 1054 cm^{-1}. MS (APCI, MeOH) 365 (M^++H).

Analysis calculated for $C_{17}H_{20}N_2O_7 \cdot 0.32\ C_3H_7OC_3H_7$: C, 57.27; H, 6.24; N, 7.04.

Found: C, 57.27; H, 6.24; N, 6.74.

3-Benzyloxycarbonylamino-5-(3,5-dioxo-10-oxa-4-aza-tricyclo[5.2.1.0^{2,6}]dec-8-en-4-yloxy)-4-oxo-pentanoic acid

Step A Prepared from 3a,4,7,7a-tetrahydro-2-hydroxy-4,7-epoxy-1H-isoindole-1,3(2H)-dione [Narita M., Teramoto T, Okawara M (Bull. Chem. Soc. Jap., 1971;44:1084)] in the manner described above, Step C, to afford 3-benzyloxycarbonylamino-5-(3,5-dioxo-10-oxa-4-aza-tricyclo[5.2.1.0^{2,6}]dec-8-en-4-yloxy)-4-oxo-pentanoic acid, 1,1-dimethylethyl ester (64%). ^{1}H NMR (400 MHz, DMSO-d_6): ä 7.84 [d, J = 8.2 Hz, 1H], 7.34 [m, 5H], 6.54 [s, 2H], 5.16 [s, 2H]; 5.07 [A of AB, J = 12.5 Hz, 1H], 5.03 [B of AB, J = 12.5 Hz, 1H], 4.93 [A of AB, J = 16.2 Hz, 1H], 4.87 [B of AB, J = 16.2 Hz, 1H], 4.52 [m, 1H], 2.87 [s, 2H], 2.73 [dd, J = 16.2, 5.8 Hz, 1H], 2.50 [obscured by dimethyl-sulfoxide (DMSO) resonance], 1.37 [s, 9H]. IR (KBr) 3421, 2979, 2930, 1790, 1726, 1520, 1368 cm^{-1}. MS (APCI, MeOH) 445 (M^+-C_4H_8).

Analysis calculated for $C_{25}H_{28}N_2O_9$: C, 59.65; H, 5.70; N, 5.35.

Found: C, 59.99; H, 5.64; N, 5.60.

Step B Prepared from 3-benzyloxycarbonylamino-5-(3,5-dioxo-10-oxa-4-aza-tricyclo[5.2.1.$0^{2,6}$]dec-8-en-4-yloxy)-4-oxo-pentanoic acid, 1,1-dimethylethyl ester in the manner described above, Step C to give 3-benzyloxy-carbonylamino-4-oxo-5-phenylacetylaminooxy-pentanoic acid (78%). IR (thin film) 3360, 1789, 1723, 1530, 1220 cm^{-1}. MS (APCI, MeOH) 445 (M^++H).

Elemental Analysis:

Calculated for $C_{21}H_{20}N_2O_9 \cdot 0.194\ CF_3CO_2H$: C, 55.06; H, 4.36; N, 5.96.

Found: C, 55.06; H, 4.58; N, 5.99.

3-Benzyloxycarbonylamino-5-(2-oxo-2,3-dihydro-indol-1-yloxy)-4-oxo-pentanoic acid

Prepared from 1-hydroxyoxindole [Kende A.S. and Thurston J. (Synthetic Communications, 1990;20:2133-8)] to give 3-benzyloxycarbonylamino-4-oxo-5-(2-oxo-2,3-dihydro-indol-1-yloxy)-pentanoic acid (24%), mp 58-70°C (decomposes).

Elemental Analysis:

Calculated for $C_{21}H_{20}N_2O_7$: C, 61.16; H, 4.89; N, 6.79.

Found: C, 60.84; H, 4.72; N, 6.46.

3-Benzyloxycarbonylamino-5-(7-methoxycarbonylmethyl-2-oxo-octahydro-indol-1-yloxy)-4-oxo-pentanoic acid

Step A Hydroxylamine hydrochloride (200 mmol, 13.8 g) was dissolved in pyridine (200 mmol, 16 mL) and methanol (10 mL), and this solution was added to a mixture of cis-2-oxo-1,3-cyclo-hexanediacetic acid, dimethyl ester [(35 mmol, 8.5 g) prepared following the procedure of Grieco P.A., Noguez J.A., Masaki Y., Hiroi K., Nishizawa M., Rosowsky A., Oppenheim S., Lazarus H. J. Med. Chem., 1977;20:71] in 200 mL of MeOH. To this solution $NaCNBH_4$ (30 mmol, 1.9 g) was added in portions over about 1 hour and the resulting solution was stirred at room temperature for 4 days. The reaction mixture was then concentrated to dryness, redissolved in 500 mL ethyl acetate, and washed 3 x 50 mL saturated NaCl, dried with Na_2SO_4, filtered, and concentrated to yield a crude solid, which was mostly desired product and pyridine.

Crude octahydro-1-hydroxy-2-oxo-1H-indole-7-acetic acid, methyl ester was recrystallized from EtOAc to yield 4.05 g (51%) of a white solid.
^{1}H-NMR: 9.26 [1H, s], 3.64 [1H, dd], 3.59 [3H, s], 2.65 [1H, dd], 2.49 [1H, dd], 2.34 [1H, dd], 2.18 [1H, m], 2.04 [1H, m], 1.79, 1H, d], 1.62 [1H, m], 1.60 [1H, s-br], 1.42 [1H, m], 1.25 [2H, m], 1.06 [1H, m]. MS (CI, NH_3) 228 (M^++H).

Step B Prepared from octahydro-1-hydroxy-2-oxo-1H-indole-7-acetic acid, methyl ester in the manner described above, Step C, to afford 3-benzyloxycarbonylamino-5-(7-methoxycarbonylmethyl-2-oxo-octahydro-indol-1-yloxy)-4-oxo-pentanoic acid, 1,1-trimethylethyl ester as a glassy oil (45%).
^{1}H NMR (400 MHz, DMSO-d_6, 1:1 mix of diastereomers): ä 7.85 [d, J = 5.8 Hz, 0.5H], 7.83 [d, J = 5.8 Hz, 0.5H], 7.35 [m, 5H], 5.06 [s, 2H], 4.94 [A of AB, J = 16.9 Hz, 0.25H], 4.87 [A of AB, J = 17.6 Hz, 0.25H], 4.82 [B of AB, J = 17.6 HZ, 0.25H], 4.74 [B of AB=, J = 16.9 Hz, 0.25H], 4.23 [m, 1H], 3.82 [m, 0.5H], 3.79 [m, 0.5H], 3.57 [s, 1.5H]; 3.57 [s, 1.5H], 2.72 [m, 0.5H], 2.70 [m, 0.5H], 2.52 [m, obscured by DMSO], 2.39 [m, 2H], 2.22 [br m, 1H], 2.10 [br m, 1H], 1.88 [br s, 0.5H], 1.84 [br s, 0.5H], 1.61 [m, 2H], 1.42 [m, 1H], 1.36 [s, 9H], 1.25 [m, 2H], 1.06 [m, 1H]. IR (thin film) 3418, 3344, 3017, 2979, 2934, 2860, 1725, 1506 cm^{-1}. MS (APCI, MeOH) 547.6 (M^++H).

Step C Prepared from 3-benzyloxycarbonylamino-5-(7-methoxycarbonylmethyl-2-oxo-octahydro-indol-1-yloxy)-4-oxo-pentanoic acid, 1,1-trimethylethyl ester in the manner described above, step D to afford 3-benzyloxy-carbonylamino-5-(7-methoxycarbonylmethyl-2-oxo-octahydro-indol-1-yloxy)-4-oxo-pentanoic acid (45%), mp 55-58°C.

^{1}H NMR (400 MHz, DMSO-d_6, 1:1 mix of diastereomers): ä 12.4 [s, 1H], 7.84 [m, 1H], 7.35 [m, 5H], 5.05 [s, 2H], 4.86 [m, 2H], 4.45 [m, 1H], 3.83 [m, 0.5H], 2.79 [m, 0.5H], 3.59 [s, 1.5H], 3.58 [s, 0.5H], 2.57 [m, obscured by DMSO], 2.41 [complex m, 4H], 2.20 [m, 2H], 1.88 [m, 1H], 1.62 [m, 2H], 1.43 [m, 2H], 1.23 [m, 2H], 1.05 [m, 1H]. IR (KBr) 3337, 2931, 1790, 1726, 15384 cm^{-1}. MS (ES, NH_4OH) 489.5 (M^+-H).

Elemental Analysis:

Calculated for $C_{24}H_{30}N_2O_9$: C, 58.77; H, 6.16; N, 5.71.

Found: C, 59.19; H, 6.40; N, 5.34.

3-Benzyloxycarbonylamino-4-oxo-5-(2-oxo-octahydro-indol-1-yloxy)-pentanoic acid

Step A Ethyl 2-cyclohexanoneacetate (4.28 g, 23.3 mmol) and O-benzyl hydroxylamine hydrochloride were combined in 100 mL of ethanol (EtOH) and 2.59 g (25.6 mmol, 3.55 mL) of triethyl amine(Et_3N) was added. The reaction was stirred at room temperature for 12 hours at which point it was concentrated in vacuo. The residue was taken up in EtOAc and washed with 1N HCl (2 x 20 mL), saturated $NaHCO_3$ (1 x 20 mL), dried over Na_2SO_4, filtered, and concentrated. Purification by chromatography (SiO_2, 90:1 hexanes-EtOAc) afforded (2-benzyloxyimino-cyclohexyl)-acetic acid ethyl ester (4.76 g, 72%) as a mixture of oxime isomers.

^1H NMR (400 MHz, $CDCl_3$ 7:1 mixture of oxime isomers): ä 7.32 [complex m, 5H], 5.05 [s, 0.25 H], 5.02 [s, 1.H], 4.05 [q, J = 7.2 Hz, 2H], 3.20 [m, 1H], 2.73 [complex m, 2H], 2.46 [d, J = 8.0 Hz, 0.125H], 2.21 [dd, J = 15.4, 6.3 Hz, 0.875H], 1.92 [m, 1H], 1.79 [complex m, 3H], 1.43 [m, 1H], 1.38 [complex m, 2H], 1.22 [t, J = 7.2 Hz, 3H]. IR (thin film) 2931, 1735, 1638, 1451 cm^{-1}. MS (CI, NH_3) 290 (M^++H).

Calculated for $C_{17}H_{23}N_1O_3$: C, 70.56; H, 8.01; N, 4.84.

Found: C, 70.47; H, 7.92; N, 4.78.

Step B (2-Benzyloxyimino-cyclohexyl)-acetic acid ethyl ester (4.66 g, 16.1 mmol) was taken up in 15 mL of acetic acid (AcOH) and $NaBH_3CN$ and stirred for 72 hours. Reaction was poured into $NaHCO_3$ and extracted into EtOAc (3 x 30 mL). The combined organic layers were washed once with brine, dried over Na_2SO_4, filtered, and concentrated. The clear oil was dissolved in 50 mL of MeOH and K_2CO_3 (5.55 g, 40.2 mmol) was added and the reaction stirred for 12 hours. The reaction was concentrated, the residue taken up in $CHCl_3$, filtered, and concentrated. Purification by chromatography (SiO_2, 4:1 hexanes/EtOAc) afforded 1.72 g (43%) of cis-(2-benzyloxyamino-cyclohexyl)-acetic acid ethyl ester and 0.441 g (11%) of trans-(2-benzyloxyamino-cyclohexyl)-acetic acid ethyl ester.

Data for cis isomer:

^{1}H NMR (400 MHz, $CDCl_3$): ä 7.44 [complex m, 2H], 7.37 [complex m, 3H], 5.05 [A of AB, J = 10.4 Hz, 1H], 4.94 [B of AB, J = 10.4 Hz, 1H], 3.47 [dd, J = 10.6, 5.3 HZ, 1H], 2.33 [dd, J = 16.4 Hz, 1H], 2.20 [m, 1H], 2.08 [dd, J = 16.4, 4.6 HZ, 1H], 1.74 [complex m, 2H], 1.60 [m, 1H], 1.32 [complex m, 5H]. IR (solution, $CHCl_3$) 3031, 2932, 2856, 1717, 1453 cm^{-1}. MS (CI, NH_3) 246 (M^++H).

Data for trans isomer: mp 79-82°C.

Elemental Analysis:

Calculated for $C_{15}H_{19}N_1O_2$: C, 73.44 H, 7.81; N, 5.71.

Found: C, 73.38; H, 7.89; N, 5.63.

Step C Prepared from cis-(2-benzyloxyamino-cyclohexyl)-acetic acid ethyl ester in the manner described above, Step B to give cis-1-hydroxy-octahydro-indol-2-one (85%), mp 85-86°C.

^{1}H NMR (400 MHz, $CDCl_3$): ä 9.86 [br s, 1H], 3.75 [dd, J = 10.4, 4.8 Hz, 1H], 2.41 [dd, J = 16.1, 7.7 Hz, 1H], 2.33 [m, 1H], 1.97 [m, 1H], 1.71 [complex m, 2H], 1.54 [m, 1H], 1.44 [complex m, 2H], 1.31 [complex m, 2H]. IR (KBr) 3037, 2936, 2856, 2710, 1690, 1659, 1548 cm^{-1}. MS (CI, NH_3) 156 (M^++H).

Elemental Analysis:

Calculated for $C_8H_{13}N_1O_2$: C, 61.91; H, 8.44; N, 9.03.

Found: C, 61.94; H, 8.49; N, 8.96.

Step D Prepared from cis-1-hydroxy-octahydro-indol-2-one in the manner described above step C to afford 3-benzyloxycarbonylamino-4-oxo-5-(2-oxo-octahydro-indol-1-yloxy)-pentanoic acid, 1,1 dimethylethyl ester (41%). IR (thin film) 2933, 1723, 1516, 1367 cm^{-1}.

Elemental Analysis:

Calculated for $C_{25}H_{134}N_2O_7$: C, 63.28; H, 7.22; N, 5.90.

Found: C, 63.03; H, 7.36; N, 5.65.

Step E Prepared from 3-benzyloxycarbonylamino-4-oxo-5-(2-oxo-octahydro-indol-1-yloxy)-pentanoic acid, 1,1- dimethylethyl ester in the manner described above, Step D to afford 3-benzyloxycarbonylamino-4-oxo-5-(2-oxo-octahydro-indol-1-yloxy)-pentanoic acid (72%). IR (KBr) 3352 (br), 2935, 2869, 1789, 1704, 1535 cm^{-1}. MS (APCI, MeOH) 419.5 (M^++H).

Elemental Analysis:

Calculated for $C_{21}H_{26}N_2O_7 \cdot 0.12\ H_2O \cdot 0.322\ CH_2Cl_2$:

C, 57.17; H, 6.05; N, 6.26.

Found: C, 57.17; H, 6.05;N, 5.89.

The following were prepared from 5-bromo-3-[2-(2-benzyloxycarbonylamino-3-methyl-butyrylamino)-propionylamino]-)-4-oxo-pentanoic acid, 1,1-dimethyl ester [Dolle R.E., et al. (J. Med. Chem., 1994;37:563-4)] in the manner described above, Step C and Step D.

3-[2-(2-Benzyloxycarbonylamino-3-methyl-butyrylamino)-propionylamino]-5-(7-methoxycarbonylmethyl-2-oxo-octahydro-indol-1-yloxy)-4-oxo-pentanoic acid

Prepared from octahydro-1-hydroxy-2-oxo-1H-indole-7-acetic acid, methyl ester (65%), mp 162-167°C, dec. Elemental Analysis:

Calculated for $C_{29}H_{34}N_4O_9 \cdot 0.75\ H_2O$ (596.127): C, 58.43; H, 6.00; N, 9.40.

Found: C, 58.40; H, 5.68; N, 9.19.

3-[2-(2-benzyloxycarbonylamino-3-methyl-butyrylamino)-propionylamino]-4-oxo-5-(2-oxo-2,3-dihydro-indol-1-yloxy)-pentanoic acid

Prepared from 1-hydroxyoxindole [Kende A.S. and Thurston J. (Synthetic Communications, 1990;20:2133-8)] to afford 3-[2-(2-benzyloxycarbonylamino-3-methyl-butyrylamino)-propionylamino]-4-oxo-5-(2-oxo-2,3-dihydro-indol-1-yloxy)-pentanoic acid(67%), mp 162-167°C, dec.

Elemental Analysis:

Calculated for $C_{29}H_{34}N_4O_9 \cdot 0.75\ H_2O$ (596.127):

C, 58.43; H, 6.00; N, 9.40.

Found: C, 58.40; H, 5.68; N, 9.19.

Other compounds were prepared using automated parallel synthesis, as follows:

To a 7-mL screw top glass vial containing 17 mg (0.3 mmol, 3 eq) of potassium fluoride was added 500 µL (0.1 mmol, 1 eq) of a 0.2 M solution of the appropriate hydroxamate in DMF. The reaction vial was agitated for a few minutes and the potassium fluoride did not completely dissolve. At this point, 500 µL (0.1 mmol, 1 eq) of 0.2 M solution of (S)-5-bromo-4-oxo-3-[[(phenylmethoxy)carbonyl]amino]-pentanoic acid, 1,1-dimethylethyl ester in DMF. The vials were capped and the rack of 30 to 40 vials were placed atop a circular agitator for 12 hours.

The reaction mixtures were diluted with 2 mL of ethyl acetate followed by 2 mL of deionized water. Two milliliters of liquid was withdrawn from the middle of the vial and injected rapidly back in twice. The vials were allowed to sit for 30 minutes and the organic layer was withdrawn from the upper half of the vial. Twice more, 2 mL of ethyl acetate was added, mixed, and separated. The combined organic layers were evaporated under a steady stream of nitrogen overnight.

The crude residue from the reactions were dissolved in 3 to 4 mL of 40% TFA in methylene chloride. The vials were agitated to ensure complete dissolution in a fume hood without caps. After 2 hours the vials were again placed under a steady stream of nitrogen overnight.

The crude reaction mixture was taken up in 1 mL of chloroform (MeOH was sometimes added to complete dissolution). The solutions were applied to 500-µ preparative silica gel TLC plates and then eluted with 20% acetone in methylene chloride with 1% to 2% acetic acid. The product bands were visualized by UV absorption, scraped from the plate, and the silica gel washed with methanol into a tared vial. The vials were placed under a stream of nitrogen overnight. The weighed purified products were then diluted to 10 mM in 25% methanol in chloroform and aliquoted to

plates for both analytical analysis and biological evaluation. The solutions were allowed to evaporate in the fume hood over 72 hours.

EXAMPLE 14: CASPASE-1 INDEPENDENT ACTIVATION OF IL-18

IL-18 is critical for the functional development of type 1 T cells and natural killer (NK) cells. Alone or in synergy with IL-12, IL-18 is a potent inducer of interferon-γ (IFNγ) from these cell types (Ushio et al. (1996) *J. Immunol.* 156:4274-4279; Okamura et al. (1995) *Nature* 378:88-91; Tomura et al. (1998) *J. Immunol.* 160:3759-3765; Takeda et al. (1998) *Immunity* 8:383-390). Caspase-1 is thought to be essential for LPS-induced activation of IL-18 and IFN-γ production (Ghayur et al. (1997) *Nature* 386:619-623; Gu et al. (1997) *Science* 275:206-209). This example shows that caspase-1 is not essential for IFN-γ production in response to non-LPS stimuli such as *E. coli*(ec)-DNA and KLH. In both these instances IL-18 is required for optimum IFNγ production. The biologically active IL-18 appears to be generated by two distinct, caspase-1 independent, mechanisms in these situations.

Il-18 is a recently cloned cytokine that shares the property of IFNγ induction with IL-12, but is structurally related to the IL-1 family of proteins (Bazan et al. (1996) *Nature* 379:591). IL-18 is synthesized as a biologically inactive precursor molecule (proIL-18) which requires proteolytic cleavage for activation. It has been shown that LPS-induced IFNγ production was dependent upon caspase-1-mediated activation to proIL-18 both *in vivo* and *in vitro*. Caspase-01-/- mice fail to generate IFNγ in response to LPS and specific caspase-1 inhibitors completely abrogate LPS-induced IFNγ production by human PBMC's. Caspases are unique intracellular cysteine proteases that require an aspartic acid residue at the P1 position, and appear to play a central role in inflammatory and apoptotic processes. Fourteen of these enzymes have been identified to date. Caspase-1 is a prototype of an inflammatory caspase that activates the pro-inflammatory cytokines, IL-1b and IL-18, in response to LPS. Caspase-3 has a different sequence specifically and is a prototype of an apoptotic caspase that cleaves and inactivated DNA repair enzymes such as PARP and DNA-PK in cells undergoing apoptosis. Biochemical studies had shown that, in addition to caspase-1, *in vitro*

transcribed and translated proIL-18 could also be processed by at least two other caspases, caspases 4 and 5. Hence, it was possible that IL-18 could be activated in cells by other mechanisms besides caspase-1 in certain situations.

To determine whether biologically active IL-18 could be generated by mechanism(s) other than caspase-1, bacterial *(E.coli,ec)*-DNA was used as a stimulus for IL-18 and IFNγ production. Ec-DNA is a bacterial product that is a potent inducer of pre-inflammatory cytokines in mammalian immune cells (Cowdrey et al. (1996) *J. Immunol.* 156:4570-4575; Sparwasser et al. (1997) *Nature* 386:336-337; Halpern et al. (1996) *Cell. Immunol.* 167:72-78). These cells response vigorously to ec-DNA by producing IFNγ, IL-12, TNF, and IL-6. Like LPS-induced IFNγ production, ec-DNA-induced IFNγ production is dependent upon IL-12. However, the Il-18 requirement for ec-DNA-induced IFNγ production was not known.

Table 3: *In Vitro* IFN-γ Production by Spleen Cells of ICE -/- and WT Mice

Treatment	IFN-γ Production by Spleen Cells of ICE -/- and WT Mice	
	ICE -/-	Wild Type
Media Alone	<20	<20
	38	<20
	52	<20
CT-DNA	54	26
	60	<20
	20	<20
Ec-DNA	2,061	1,622
	2,425	2,512
	1,970	1,044

Experiments were performed to determine if ec-DNA-induced IFNγ production required IL-18 and if this IL-18 production was caspase-dependent. First, it was determined if caspase-1 deficient mice made IFNγ in response to ec-DNA. Caspase-1-/- and wild-type (WT) mice were primed with *P. acnes* and then challenged with ec-DNA in vivo. Two hours after ec-DNA challenge, serum was collected and IFNγ titers were determined by an ELISA (Figure 7). The results in Figure 7 demonstrate that caspase-1-/- mice made as much IFNγ as WT mice in response to ec-DNA. Furthermore, both caspase-1-/- and WT mice showed similar levels of serum TNF, but IL-1β was undetectable in the serum in either genotype under these conditions. Similarly, *P. acnes*-primed splenocytes from caspase-1-/- and WT mice produced IFNγ (and TNF, but not IL-1β) following stimulation with c-DNA. However, no cytokine production was induced by mammalian (calf thymus DNA, ct-DNA) (Table 3) confirming earlier observations (Cowdrey et al. (1996) *J. Immunol.* 156:4570-4575; Sparwasser et al. (1997) *Nature* 386:336-337). These results indicate that ec-DNA-induced IFNγ

production may not require caspase-1. It has been hypothesized that mammalian immune cells may respond specifically to bacterial DNA in an ICE-independent manner.

The results in Figure 7 and Table 3 raised two possibilities: (i) ec-DNA-induced IFNγ production could be dependent upon Il-12, but not IL-18 since caspase-1-/- mice make normal levels of IL-12 (Ghayur et al. (1997) *Nature* 386:619-623); (ii) both IL-12 and IL-18 may be necessary for ec-DNA-induced IFNγ production, but unlike after LPS stimulation, a caspase(s) other than caspase-1, is required for ec-DNA-induced IL-18 and IFNγ production.

To distinguish between these two possibilities, the dependence of ec-DNA-induced IFNγ production on IL-12 and/or IL-18 was investigated (Figure 8). Addition of an anti-mouse IL-12 monoclonal antibody (C17.15) almost completely abrogated IFNγ production from ecDNA-stimulated caspase-1-/- splenocytes. Addition of rIL-12 to these cultures restored IFNγ production. Similarly, addition of neutralizing anti-mouse IL-18 antiserum to ec-DNA stimulated caspase-1-/- splenocytes resulted in over 70% inhibition of IFNγ production. In addition, spleen cells from IL-18-/- mice failed to produce IFNγ production by Il-18-/- spleen cells. These results may demonstrate that, in addition to IL-12, ec-DNA-induced IFNγ production was also dependent upon IL-18. More importantly, since the spleen cells of caspase-1-/- mice could generate biologically active IL-18 ( ), the results suggest that ec-DNA-induced IL-18 activation and thereby IFNγ production was independent of caspase-1.

Next, it was determined that ec-DNA-induced, IFNγ production by caspase-1-/- spleen cells was dependent on caspase other than caspase-1. Spleen cells from *P. acnes*-primed caspase-1-/- mice were cultured with ec-DNA in the presence of absence of a caspase-1 specific inhibitor (Ac-YVAD-CHO, (Thornberry et al. (1992) *Nature* 356:768-774)), a caspase-3 specific inhibitor (DEVD-CHO, (Nicholson et al. (1995) *Nature* 376:37-43)), or a pan caspase inhibitor (z-VAD-FMK, (Miwa et al. (1998) *Nature Medicine* 4:1287-1292)). Supernatants were collected 18-20h later and IFNγ levels were determined (Figure 9). Neither the caspase-1-specific inhibitor nor the caspase-3 specific inhibitor blocked ec-DNA-induced IFNγ production by the spleen cells. In contrast, the psn-caspase inhibitor, z-VAD-FMK, almost completely abrogated ec-DNA-induced IFNγ production by spleen cells of caspase-1-/- mice. Similar results

were observed with spleen cells from WT mice. Addition of rIL-18 completely restored IFNγ production from caspase-1-/- spleen cells cultured with ec-DNA in the presence of z-VAD-FMK (Figure 9). These data suggested that ec-DNA-induced IFN-γ production was dependent upon a caspase other than caspase-1 or caspase3. Collectively, these results confirm that IL-18 absolutely required for ec-DNA-induced IFN-γ production, and that IL-18 can be generated by a caspase other than caspase-1 or caspase-3 like-protease.

The role of caspases in IFNγ production from T cells was also studied. Caspase-1-/- mice immunized with KLH were found to generate normal levels of IFNγ following stimulation of draining lymph node lymphocytes with KLH *ex vivo* (Figure 10a). To determine the IL-18-dependence of KLH-induced IFNγ production, the effects of anti-IL-18 antiserum or anti-IL-12 antibodies on this response were studied. Separately, anti-IL-18 or anti-IL-12 antibodies inhibited KLH-induced IFNγ production only partially (-50%) showing that each of these cytokines was important for optimal IFNγ production after T cell activation. However, the combination of anti-IL-18 and anti-IL-12 antibodies resulted in >80% inhibition of KLH-induced IFNγ production (Figure 10a) These results were similar to those recently observed in IL-12-I, IL-18-/-, and Il-12-/-IL-18-/- mice.

The requirement of caspases in the generation of IFNγ from T cells was also investigated, by using pan-caspase inhibitor, Z-VZD-FMK. The inhibitor failed to inhibit IFNγ production from KLH-primed T cells (Figure 10b). These results suggested that either z-VAD-FMK does not inhibit all caspases in this context or that IL-18 production during T cell activation I mediated by a caspase-independent mechanism(s).

Caspase-1 -/- mice are defective in LPS induced, but not ec-DNA-induced IFNγ production as shown in Figures 11a and 11b.

In Figures 12a, 12b and 12c, it is shown that ec-DNA-induced IFNγ production is inhibited by a pan-caspase inhibitor (z-VAD-FMK), but not by the caspase-1 inhibitor (YVAD-CHO), or the caspase-3 inhibitor (DEVD-CHO). Figure 12a shows the IFN-γ production using wild type mice. Figure 12b and 12c show the IFN-γ production using caspase-1 -/- mice.

In addition to stimulating IFN-γ production, IL-18 induces several other pro-inflammatory cytokines (Horwood et al. (1998) *J. Clin. Invest.* 101:595-603; Puren et al. (1998) *J. Clin. Invest.* 101:711-721; Udagawa et al. (1997) *J. Exp. Med.* 185:1005-1012) and mediators of inflammation (Kohka et al. (1998) *J. Leukoc. Biol.* 64:519-527; Olee et al. (1999) *J. Immunol.* 162:1096-1100). Recent studies in humans and animal models of human disease suggest involvement of IL-18 in the pathogenesis of acute myelogenous leukemias, (Taniguchi et al. (1997) *J. Immunol Met.* 206:107-113), HIV activation, sepsis/septic shock (Tsutsui et al. (1997) *J. Immunol.* 159:3961-3967), degenerative liver diseases (Wildbaum et al. (1998) *J. Immunol.* 161:6368-6374), insulin-dependent diabetes milleyus (Rothe et al. (1997) *J. Clin. Invest.* 99:469), multiple sclerosis (Xu et al. (1998) *J. Exp. Med.* 188:1485-1492), and localized inflammatory processes. Thus, IL-18 is fast emerging as a key pro-inflammatory cytokine. These observations offer several therapeutic options to regulate the production or functions of biologically active IL-18. Inhibition of specific caspases that regulate generation of bioactive IL-18 would be useful in situations where the putative caspase(s) involved was known, e.g. after LPS or ecDNA stimulation. However, in inflammatory situations where caspases may not play a dominant role in IL-18 maturation such as after T cell activation, the enzyme necessary for proteolytic activation of IL-18 needs to be delineated for development of small molecule inhibitors of IL-18 production.

Materials and Methods

In vivo ec-DNA-induced IFNγ production: Caspase-1-/- and WT mice were injected i.p. with 1 mg of killed *P. acnes* bacteria. Seven days following this priming, mice were injected i.v. with 0.3 mg of purified *E.coli*-DNA. Two hours later the mice were sacrificed and blood collected by cardiac puncture. Serum titers of IFNγ, TNF and IL-1β were determined by specific ELISA kits (R&D Systems)

In vitro ec-DNA induced IFNγ production: Single cell suspensions of individual (Table 3) or pooled spleens (9-10 spleens, Figures 9-12) from caspase-1-/- mice, or IL-18-/- mice and WT mice were prepared in RPMI-1640 medium supplemented with 10% FCS

(complete medium). Splenocytes were plated at 20 X 10^6 cells/ml in 6-well plates and 25 μg of ec-DNA added/well. In some experiments, anti-IL-12 monoclonal antibody (C17.15, G. Trinchieri) ± recombinant IL-12, or anti-IL-18 serum (source) ± recombinant IL-18, or caspase inhibitors ± recombinant IL-18 were added to cultures stimulated with ec-DNA. The cells were cultured overnight and supernatants were collected. IFNγ titers in the supernatants were determined by a murine IFNγ specific ELISA kit.

KLH-induced IFNγ production: Caspase-1-/- or WT mice (6-7 mice/group) were injected s.c. with 100μ of keyhole, limpet hemocyanin (KLH) emulsified in CFA. Four days later, mice were sacrificed and the draining lymph nodes were excised. Single cell suspensions of lymph nodes lymphocytes were prepared and 1X10^6/well cells were plated in 96-well plates, and cultured with 25 μ/ml of KLH or medium. In some experiments, anti-IL-12 monoclonal antibody (C17.15) or anti-IL-18 serum (source), or caspase inhibitors were added to the cultures. The cells were cultured for 48 hours and supernatants collected for IFNγ estimation by ELISA.

EQUIVALENTS

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

CLAIMS

1. A method for modulating responsiveness to a corticosteroid in a subject, comprising administering to the subject:

an agent which antagonizes a target that regulates production of interferon-γ (IFN-γ) in the subject, the agent being administered at a dosage and by a route sufficient to inhibit production of IFN-γ in the subject; and

a corticosteroid,

such that responsiveness of the subject to the corticosteroid is modulated as compared to when a corticosteroid alone is administered to the subject.

2. The method of claim 1, wherein the agent is an IL-18 antagonist, the agent being administered at a dosage and by a route sufficient to inhibit IL-18 activity in the subject.

3. The method of claim 2, wherein the agent blocks caspase-1 activation of IL-18.

4. The method of claim 3, further comprising administering at least one second agent which blocks caspase-1 independent activation of IL-18.

5. The method of claim 4, wherein said second agent is an inhibitor of caspase-4.

6. The method of claim 4, wherein said second agent is an inhibitor of caspase-5.

7. The method of claim 4, wherein said second agent is an inhibitor of PR3.

8. The method of claim 2, wherein the agent is an antibody, antibody fragment, a natural or native binding protein, or an engineered binding protein that binds IL-18 or an IL-18 receptor.

9. The method of claim 8, wherein the antibody is a chimeric, humanized or human antibody.

10. The method of claim 8, wherein the antibody is a neutralizing antibody.

11. The method of claim 8, wherein the antibody is a monoclonal anti-IL-18 antibody.

12. The method of claim 1, wherein the agent is an interleukin-12 (IL-12) antagonist, the agent being administered at a dosage and by a route sufficient to inhibit IL-12 activity in the subject.

13. The method of claim 12, wherein the agent is an antibody, antibody fragment, a natural or native binding protein, or a engineered binding protein that binds IL-12 or IL-12 receptor.

14. The method of claim 13, wherein the antibody is a chimeric, humanized, or human antibody.

15. The method of claim 13, wherein the antibody is a neutralizing antibody.

16. The method of claim 13, wherein the antibody is a monoclonal anti-IL-12 antibody.

17. The method of claim 12, wherein the agent stimulates cyclic AMP production in cells that produce IL-12.

18. The method of claim 12, wherein the agent is a phosphodiesterase IV inhibitor.

19. The method of claim 18, wherein the phosphodiesterase IV inhibitor is selected from the group consisting of 4-arylpyrrolidinones, rolipram, denbufylline, tibenelast, nitraquazone, CP-80633, quinazolinediones and CP-77059.

20. The method of claim 19, wherein the agent is a beta-2 agonist.

21. The method of claim 20, wherein the beta-2 agonist is selected from the group consisting of salmeterol, fenoterol and isoproterenol.

22. The method of claim 12, wherein the agent is a STAT4 inhibitor.

23. The method of claim 1, wherein the agent is an anti-IL-1α antibody, an anti-IL-1β antibody, or an anti-TNF antibody.

24. The method of claim 1, wherein the agent is an NK cell antagonist.

25. The method of claim 24, wherein the agent is an anti-NK/NK-like cell antibody.

26. The method of claim 1, wherein the agent is a T-cell antagonist.

27. The method of claim 26, wherein said T-cell antagonist is an anti-T-cell antibody, or an antibody to a T-cell activation marker.

28. The method of claim 1, wherein the corticosteroid is selected from the group consisting of cortisone, hydrocortisone, beclomethasone, flunisolide, prednisone, prednisolone, methylprednisolone, triamcinolone, deflazacort, betamethasone and dexamethasone.

29. The method of claim 1, wherein the subject is suffering from an autoimmune disease or disorder.

30. The method of claim 1, wherein the subject is suffering from an acute or chronic inflammatory disorder.

31. The method of claim 1, wherein the subject is suffering from an immunoinflammatory disease or disorder selected from the group consisting of asthma, adult respiratory distress syndrome, systemic lupus erythematosus, inflammatory bowel disease, Crohn's disease, ulcerative colitis, multiple sclerosis, insulin-dependent diabetes mellitus, autoimmune arthritis, rheumatoid arthritis, juvenile rheumatoid arthritis, psoriatic arthritis, inflammatory pulmonary syndrome, pemphigus vulgaris, idiopathic thrombocytopenic purpura, autoimmune meningitis, myasthenia gravis, autoimmune thyroiditis, dermatitis, atopic dermatitis, eczematous dermatitis, psoriasis, Sjögren's Syndrome, keratoconjunctivitis sicca secondary to Sjögren's Syndrome, alopecia areata, allergic responses due to arthropod bite reactions, aphthous ulcer, iritis, conjunctivitis, keratoconjunctivitis, cutaneous lupus erythematosus, scleroderma, vaginitis, proctitis, drug eruptions, Stevens-Johnson syndrome, leprosy reversal reactions, erythema nodosum leprosum, autoimmune uveitis, allergic encephalomyelitis, aplastic anemia, pure red cell anemia, idiopathic thrombocytopenia, polychondritis, Wegener's granulomatosis, chronic active hepatitis, Graves ophthalmopathy, primary biliary cirrhosis, uveitis posterior and interstitial lung fibrosis.

32. The method of claim 1, wherein the subject is suffering from septic shock or sepsis, Crohn's disease, asthma, graft versus host disease or transplant rejection, complications associated with post-surgical stress, Still's disease, leukemia, or rheumatoid arthritis.

33. The method of claim 1, wherein steroid resistance in the subject is reversed, as compared to when a corticosteroid alone is administered to the subject

34. The method of claim 1, wherein steroid sensitivity in the subject is increased, as compared to when a corticosteroid alone is administered to the subject.

35. The method of claim 1, wherein the corticosteroid is administered to the subject according to a schedule that tapers the dosage of the corticosteroid over time

36. The method of claim 35, wherein the method ameliorates a steroid rebound effect associated with administration of reduced dosages of the corticosteroid.

37. The method of claim 35, wherein the dosage of the corticosteroid is tapered to zero.

38. A method for modulating responsiveness to corticosteroids in a subject, comprising administering to the subject:

at least one agent that is an inhibitor of a caspase family protease; and

a corticosteroid,

such that responsiveness of the subject to the corticosteroid is modulated as compared to when a corticosteroid alone is administered to the subject.

39. The method of claim 38, wherein the corticosteroid is selected from the group consisting of cortisone, hydrocortisone, beclomethasone, flunisolide, prednisone, prednisolone, methylprednisolone, triamcinolone, deflazacort, betamethasone and dexamethasone.

40. The method of claim 38, wherein the subject is suffering from an autoimmune disease or disorder.

41. The method of claim 38, wherein the subject is suffering from an acute or chronic inflammatory disorder.

42. The method of claim 38, wherein the subject is suffering from an immunoinflammatory disease or disorder selected from the group consisting of asthma, adult respiratory distress syndrome, systemic lupus erythematosus, inflammatory bowel disease, Crohn's disease, ulcerative colitis, multiple sclerosis, insulin-dependent diabetes mellitus, autoimmune arthritis, rheumatoid arthritis, juvenile rheumatoid arthritis, psoriatic arthritis, inflammatory pulmonary syndrome, pemphigus vulgaris, idiopathic thrombocytopenic purpura, autoimmune meningitis, myasthenia gravis, autoimmune thyroiditis, dermatitis, atopic dermatitis, eczematous dermatitis, psoriasis, Sjögren's Syndrome, keratoconjunctivitis sicca secondary to Sjögren's Syndrome, alopecia areata, allergic responses due to arthropod bite reactions, aphthous ulcer, iritis, conjunctivitis, keratoconjunctivitis, cutaneous lupus erythematosus, scleroderma, vaginitis, proctitis, drug eruptions, Stevens-Johnson syndrome, leprosy reversal reactions, erythema nodosum leprosum, autoimmune uveitis, allergic encephalomyelitis, aplastic anemia, pure red cell anemia, idiopathic thrombocytopenia, polychondritis, Wegener's granulomatosis, chronic active hepatitis, Graves ophthalmopathy, primary biliary cirrhosis, uveitis posterior and interstitial lung fibrosis.

43. The method of claim 38, wherein the subject is suffering from septic shock or sepsis, Crohn's disease, asthma, graft versus host disease or transplant rejection, complications associated with post-surgical stress, Still's disease, leukemia, or rheumatoid arthritis.

44. The method of claim 38, wherein steroid resistance in the subject is reversed, as compared to when a corticosteroid alone is administered to the subject

45. The method of claim 38, wherein steroid sensitivity in the subject is increased, as compared to when a corticosteroid alone is administered to the subject.

46. The method of claim 38, wherein the corticosteroid is administered to the subject according to a schedule that tapers the dosage of the corticosteroid over time.

47. The method of claim 46, wherein the method ameliorates a steroid rebound effect associated with administration of reduced dosages of the corticosteroid.

48. The method of claim 46, wherein the dosage of the corticosteroid is tapered to zero.

49. A method for modulating responsiveness to corticosteroids in a subject, comprising administering to the subject:

an agent that is an antagonist of interleukin-12 (IL-12), the agent being administered at a dosage and by a route sufficient to inhibit IL-12 activity in the subject; and

a corticosteroid,

such that responsiveness of the subject to the corticosteroid is modulated as compared to when a corticosteroid alone is administered to the subject.

50. The method of claim 49, wherein the agent is an antibody, antibody fragment, natural or native binding protein or engineered binding protein that binds IL-12 or IL-12 receptor.

51. The method of claim 50, wherein the agent is an anti-IL-12 monoclonal antibody.

52. The method of claim 50, wherein the agent is a chimeric, humized or human antibody.

53. The method of claim 50, wherein the agent is a neutralizing antibody.

54. The method of claim 49, wherein the corticosteroid is selected from the group consisting of cortisone, hydrocortisone, beclomethasone, flunisolide, prednisone, prednisolone, methylprednisolone, triamcinolone, deflazacort, betamethasone and dexamethasone.

55. The method of claim 49, wherein the subject is suffering from an autoimmune disease or disorder.

56. The method of claim 49, wherein the subject is suffering from an acute or chronic inflammatory disorder.

57. The method of claim 49, wherein the subject is suffering from an immunoinflammatory disease or disorder selected from the group consisting of asthma, adult respiratory distress syndrome, systemic lupus erythematosus, inflammatory bowel disease, Crohn's disease, ulcerative colitis, multiple sclerosis, insulin-dependent diabetes mellitus, autoimmune arthritis, rheumatoid arthritis, juvenile rheumatoid arthritis, psoriatic arthritis, inflammatory pulmonary syndrome, pemphigus vulgaris, idiopathic thrombocytopenic purpura, autoimmune meningitis, myasthenia gravis, autoimmune thyroiditis, dermatitis, atopic dermatitis, eczematous dermatitis, psoriasis, Sjögren's Syndrome, keratoconjunctivitis sicca secondary to Sjögren's Syndrome, alopecia areata, allergic responses due to arthropod bite reactions, aphthous ulcer, iritis, conjunctivitis, keratoconjunctivitis, cutaneous lupus erythematosus, scleroderma, vaginitis, proctitis, drug eruptions, Stevens-Johnson syndrome, leprosy reversal reactions, erythema nodosum leprosum, autoimmune uveitis, allergic encephalomyelitis, aplastic anemia, pure red cell anemia, idiopathic thrombocytopenia, polychondritis, Wegener's granulomatosis, chronic active hepatitis, Graves ophthalmopathy, primary biliary cirrhosis, uveitis posterior and interstitial lung fibrosis.

58. The method of claim 49, wherein the subject is suffering from septic shock or sepsis, Crohn's disease, asthma, graft versus host disease or transplant rejection, complications associated with post-surgical stress, Still's disease, leukemia, or rheumatoid arthritis.

59. The method of claim 49, wherein steroid resistance in the subject is reversed, as compared to when a corticosteroid alone is administered to the subject

60. The method of claim 49, wherein steroid sensitivity in the subject is increased, as compared to when a corticosteroid alone is administered to the subject.

61. The method of claim 49, wherein the corticosteroid is administered to the subject according to a schedule that tapers the dosage of the corticosteroid over time.

62. The method of claim 61, wherein the method ameliorates a steroid rebound effect associated with administration of reduced dosages of the corticosteroid.

63. The method of claim 62, wherein the dosage of the corticosteroid is tapered to zero.

64. A method for modulating responsiveness to corticosteroids in a subject, comprising administering to the subject:

an NK cell antagonist, the NK cell antagonist being administered at a dosage and by a route sufficient to inhibit IFN-γ activity in the subject; and

a corticosteroid,

such that responsiveness of the subject to the corticosteroid is modulated as compared to when a corticosteroid alone is administered to the subject.

65. The method of claim 64, wherein the agent is an antibody, antibody fragment, or engineered binding protein that binds to an NK/NK-like cell surface marker.

66. The method of claim 65, wherein the agent is an anti-NK/NK-like cell antibody.

67. The method of claim 66, wherein the antibody is an anti-asialo-GM1 antibody or an NK1.1 antibody.

68. The method of claim 64, wherein the subject is suffering from an autoimmune disease or disorder.

69. The method of claim 64, wherein the subject is suffering from an acute or chronic inflammatory disorder.

70. The method of claim 64, wherein the subject is suffering from an immunoinflammatory disease or disorder selected from the group consisting of asthma, adult respiratory distress syndrome, systemic lupus erythematosus, inflammatory bowel disease, Crohn's disease, ulcerative colitis, multiple sclerosis, insulin-dependent diabetes mellitus, autoimmune arthritis, rheumatoid arthritis, juvenile rheumatoid arthritis, psoriatic arthritis, inflammatory pulmonary syndrome, pemphigus vulgaris, idiopathic thrombocytopenic purpura, autoimmune meningitis, myasthenia gravis, autoimmune thyroiditis, dermatitis, atopic dermatitis, eczematous dermatitis, psoriasis, Sjögren's Syndrome, keratoconjunctivitis sicca secondary to Sjögren's Syndrome, alopecia areata, allergic responses due to arthropod bite reactions, aphthous ulcer, iritis, conjunctivitis, keratoconjunctivitis, cutaneous lupus erythematosus, scleroderma, vaginitis, proctitis, drug eruptions, Stevens-Johnson syndrome, leprosy reversal reactions, erythema nodosum leprosum, autoimmune uveitis, allergic encephalomyelitis, aplastic anemia, pure red cell anemia, idiopathic thrombocytopenia, polychondritis, Wegener's granulomatosis, chronic active hepatitis, Graves ophthalmopathy, primary biliary cirrhosis, uveitis posterior and interstitial lung fibrosis.

71. The method of claim 64, wherein the subject is suffering from septic shock or sepsis, Crohn's disease, asthma, graft versus host disease or transplant rejection, complications associated with post-surgical stress, Still's disease, leukemia, or rheumatoid arthritis.

72. The method of claim 64, wherein steroid resistance in the subject is reversed, as compared to when a corticosteroid alone is administered to the subject

73. The method of claim 64, wherein steroid sensitivity in the subject is increased, as compared to when a corticosteroid alone is administered to the subject.

74. The method of claim 64, wherein the corticosteroid is administered to the subject according to a schedule that tapers the dosage of the corticosteroid over time.

75. The method of claim 74, wherein the method ameliorates a steroid rebound effect associated with administration of reduced dosages of the corticosteroid.

76. The method of claim 74, wherein the dosage of the corticosteroid is tapered to zero.

77. A method for modulating responsiveness to a corticosteroid in a subject, comprising:

selecting a subject in need of modulation of responsiveness to a corticosteroid; and

administering to the subject a corticosteroid and an agent which antagonizes a target that regulates production of interferon-γ (IFN-γ) in the subject, the agent being administered at a dosage and by a route sufficient to inhibit production of IFN-γ in the subject,

such that responsiveness of the subject to a corticosteroid is modulated as compared to when a corticosteroid alone is administered to the subject.

78. The method of claim 77, wherein the subject is resistant to a corticosteroid prior to administration of the agent.

79. The method of claim 77, wherein the subject is responsive to a corticosteroid prior to administration of the agent but exhibits increased sensitivity to the corticosteroid after administration of the agent.

80. The method of claim 77, wherein treatment of the subject with a corticosteroid is to be stopped and administration of the agent ameliorates a steroid rebound effect in the subject.

81. The method of claim 77, wherein the agent is an IL-18 antagonist, the agent being administered at a dosage and by a route sufficient to inhibit IL-18 activity in the subject.

82. The method of claim 77, wherein the agent is an Interleukin-12 (IL-12) antagonist, the agent being administered at a dosage and by a route sufficient to inhibit IL-12 activity in the subject.

83. A method for modulating responsiveness to a corticosteroid in a subject, comprising administering to the subject:

an agent which antagonizes a target that regulates production of interferon-γ (IFN-γ) in the subject, and

a corticosteroid,

such that responsiveness of the subject to the corticosteroid is modulated.

84. The method of claim 83, wherein said agent is an IL-18 antagonist.

85. The method of claim 83, wherein said agent is an IL-12 antagonist.

86. A method for regulating the production of IFN-γ in a subject, comprising administering to the subject:

an agent which antagonizes a target that regulates production of IFN-γ in the subject; and

a corticosteroid,

such that production of INF-γ is modulated in the subject.

87. The method of claim 86, wherein said agent is an IL-18 antagonist.

88. The method of claim 86, wherein said agent is an IL-12 antagonist.

89. The method of claims 8, 11, 13, 16, 83, or 86, wherein the agent or the corticosteroid is administered in a pharmaceutically acceptable carrier.

90. The method of claims 8, 11, 13, 16, 83, or 86, wherein the agent and the corticosteroid are administered separately or together in one or more pharmaceutically acceptable carriers.

91. A pharmaceutical composition comprising an agent which antagonizes a target that regulates production of interferon-γ (IFN-γ) in the subject, a corticosteroid and a pharmaceutically acceptable carrier.

92. The pharmaceutical composition of claim 91, wherein the agent is an inhibitor of a caspase family protease, an anti-IL-12 monoclonal antibody or an anti-IL-18 monoclonal antibody.

93. The pharmaceutical composition of claim 91 which is formulated for topical, intrarectal, inhalation, intravenous, intramuscular, or subcutaneous administration.

FIG.1

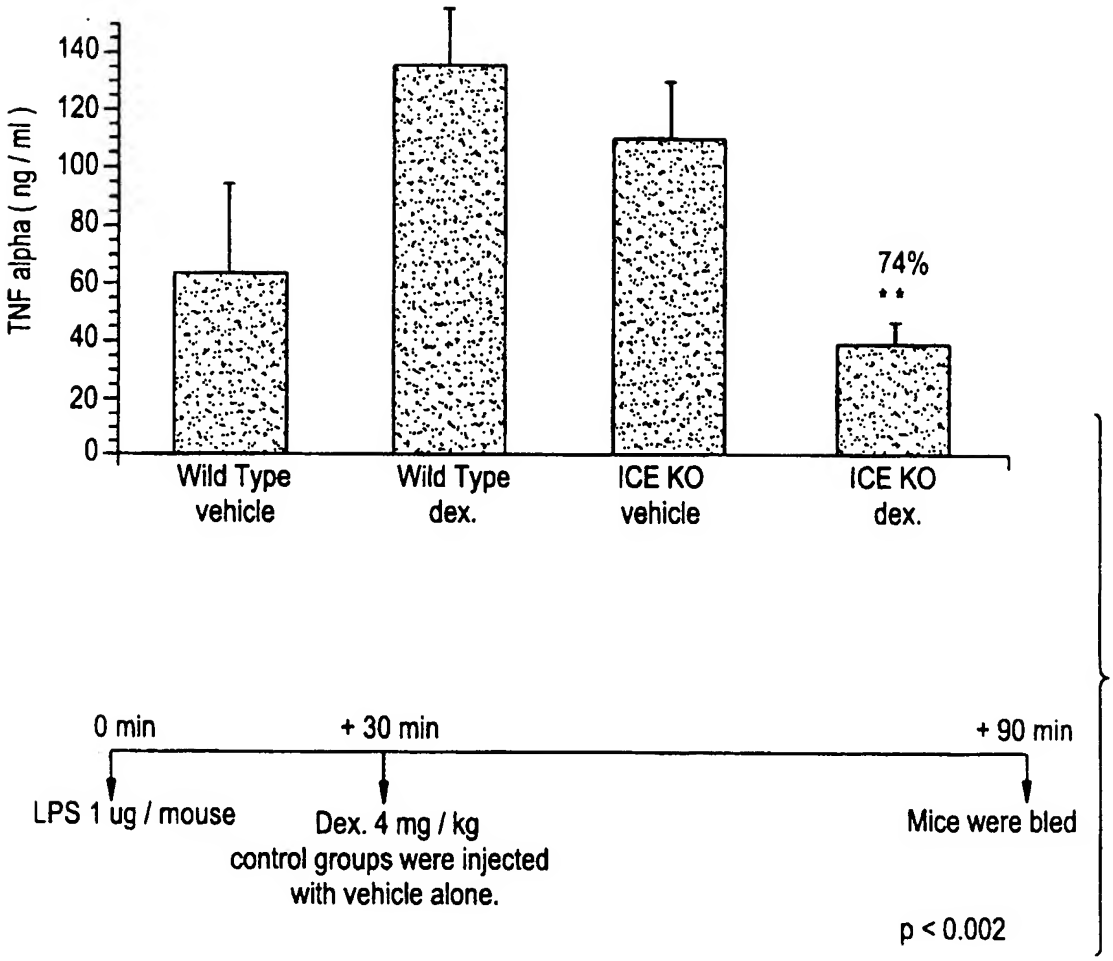

TNF alpha (ng / ml)
140
120
100
80
60
40
20
0
74%
**
Wild Type vehicle
Wild Type dex.
ICE KO vehicle
ICE KO dex.
0 min
+ 30 min
+ 90 min
LPS 1 ug / mouse
Dex. 4 mg / kg
control groups were injected with vehicle alone.
Mice were bled
p < 0.002

FIG.2

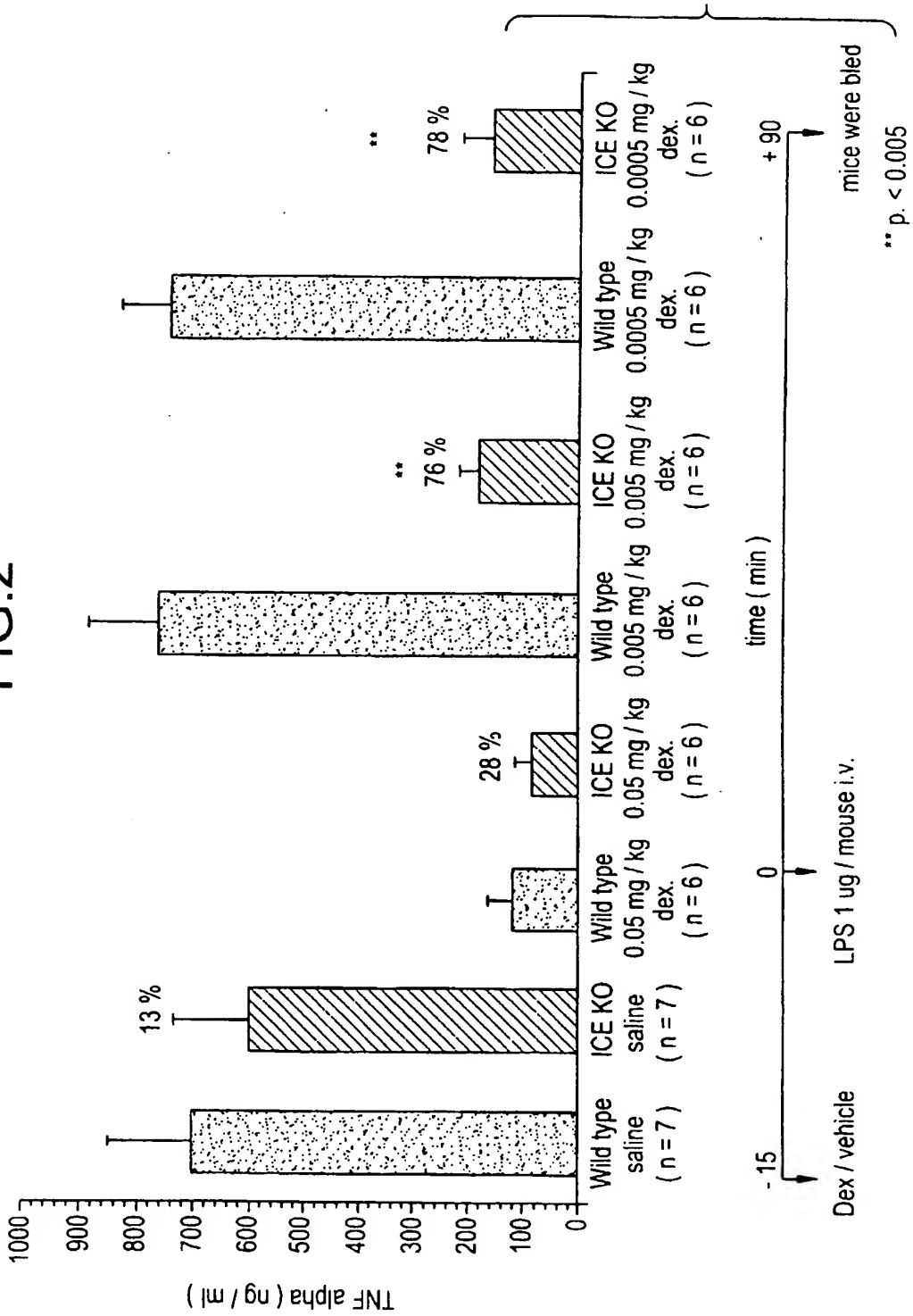

TNF alpha (ng / ml)
1000
900
800
700
600
500
400
300
200
100
0
13 %
28 %
76 %
78 %
**
**
Wild type saline (n = 7)
ICE KO saline (n = 7)
Wild type 0.05 mg / kg dex. (n = 6)
ICE KO 0.05 mg / kg dex. (n = 6)
Wild type 0.005 mg / kg dex. (n = 6)
ICE KO 0.005 mg / kg dex. (n = 6)
Wild type 0.0005 mg / kg dex. (n = 6)
ICE KO 0.0005 mg / kg dex. (n = 6)
- 15
0
+ 90
time (min)
Dex / vehicle
LPS 1 ug / mouse i.v.
mice were bled
** p. < 0.005

FIG.3

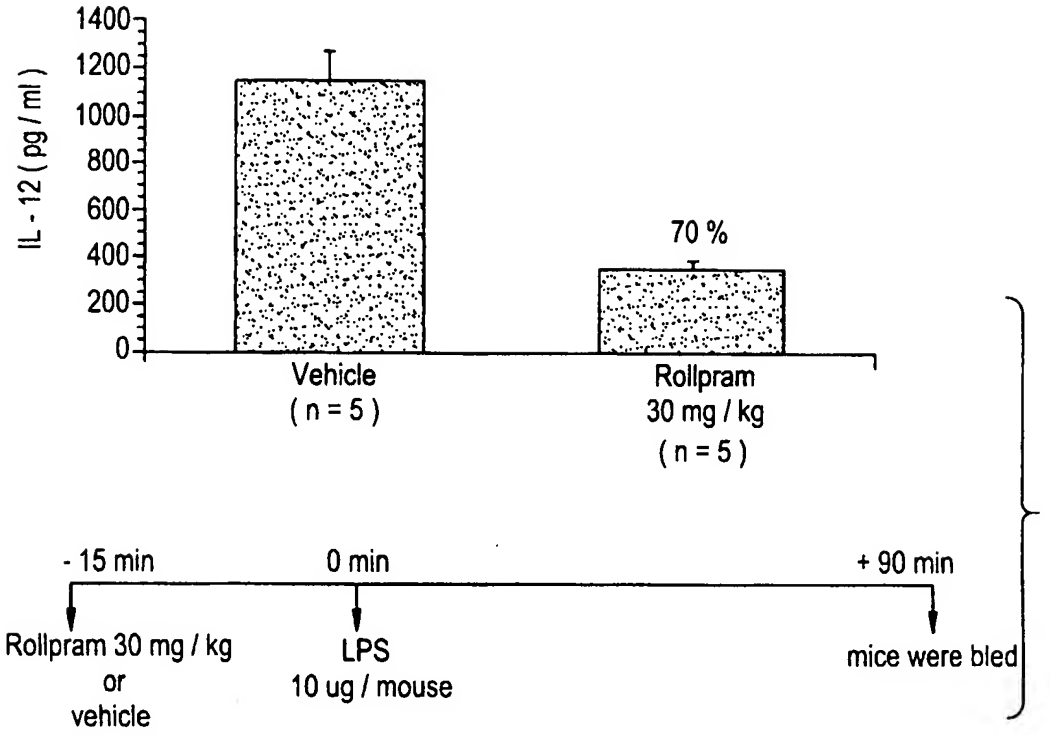

IL - 12 (pg / ml)
1400
1200
1000
800
600
400
200
0
70 %
Vehicle
(n = 5)
Rollpram
30 mg / kg
(n = 5)
- 15 min
0 min
+ 90 min
Rollpram 30 mg / kg
or
vehicle
LPS
10 ug / mouse
mice were bled

FIG.4

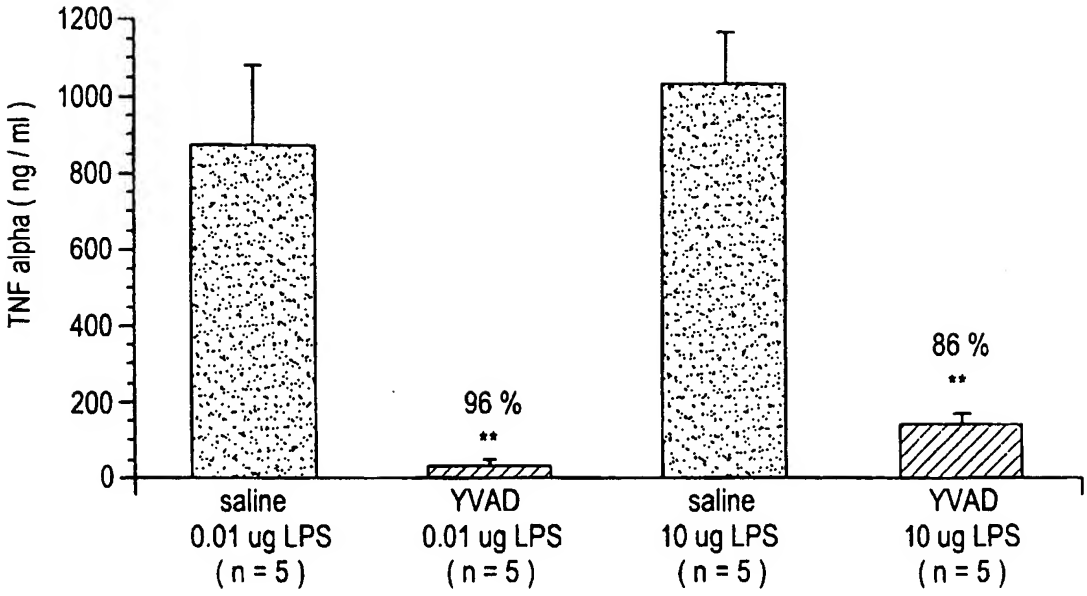

TNF alpha (ng / ml)
1200
1000
800
600
400
200
0
96 %
**
86 %
**
saline
0.01 ug LPS
(n = 5)
YVAD
0.01 ug LPS
(n = 5)
saline
10 ug LPS
(n = 5)
YVAD
10 ug LPS
(n = 5)

FIG.5

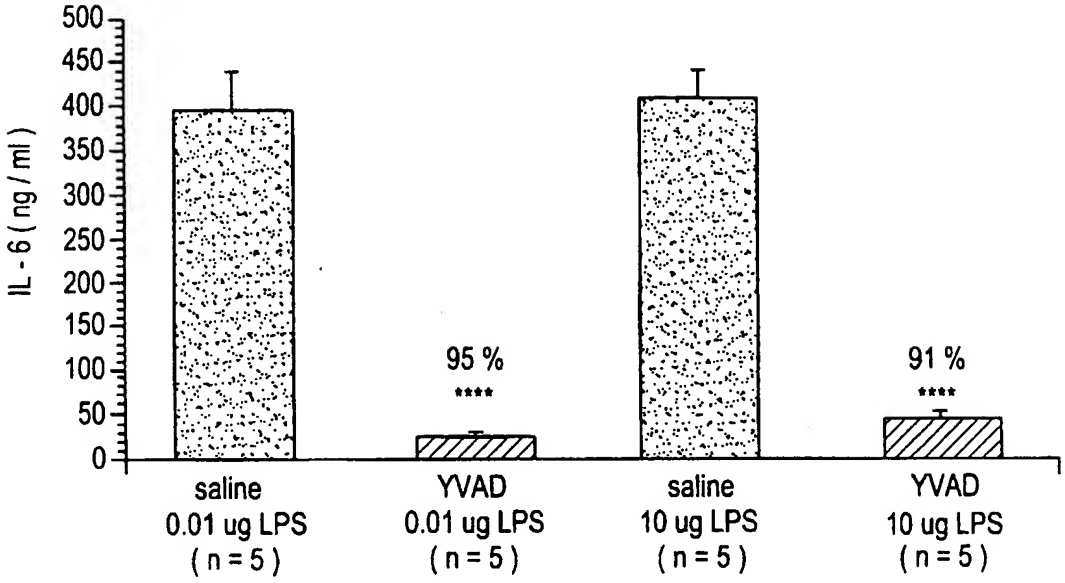
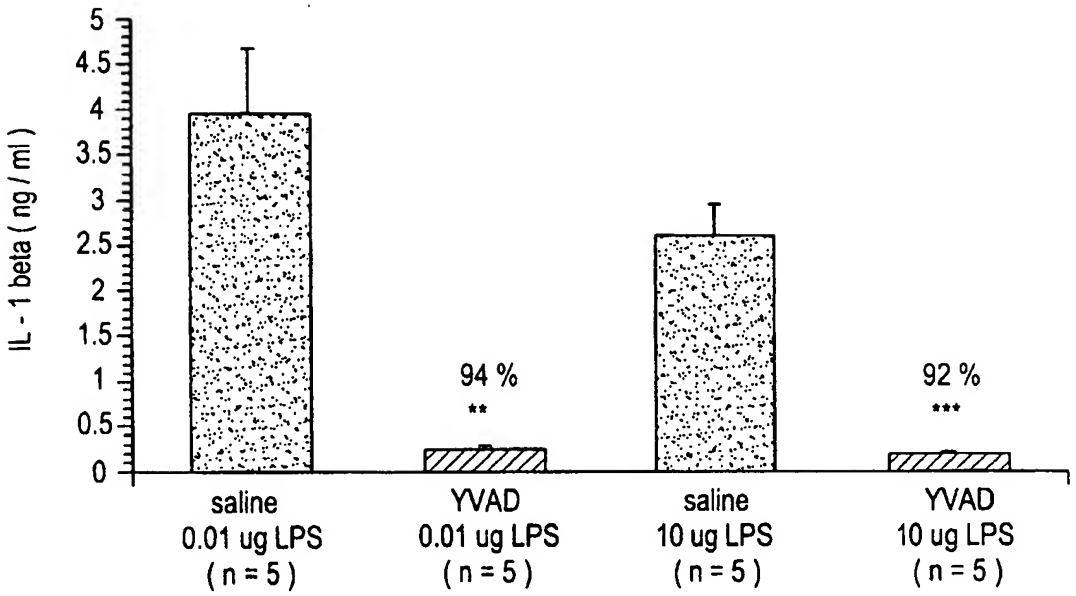

IL-6 (ng/ml)
500
450
400
350
300
250
200
150
100
50
0
95 %
****
91 %
****
saline
0.01 ug LPS
(n = 5)
YVAD
0.01 ug LPS
(n = 5)
saline
10 ug LPS
(n = 5)
YVAD
10 ug LPS
(n = 5)

FIG.6


IL - 1 beta (ng / ml)
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
94 %
**
92 %
***
saline
0.01 ug LPS
(n = 5)
YVAD
0.01 ug LPS
(n = 5)
saline
10 ug LPS
(n = 5)
YVAD
10 ug LPS
(n = 5)

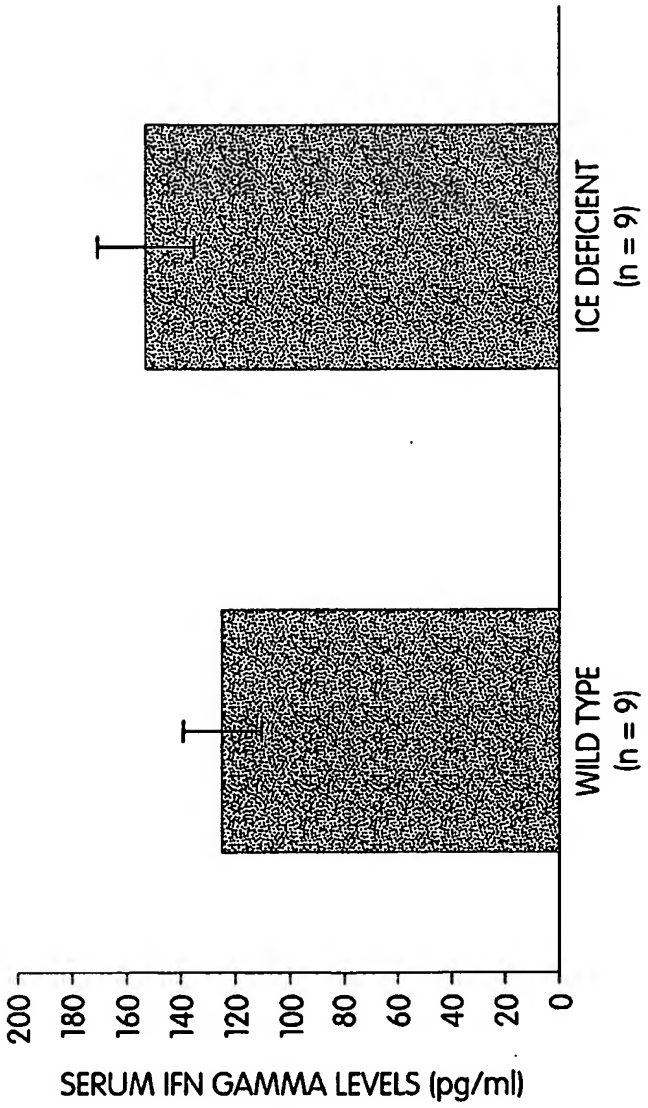

ICE DEFICIENT
(n = 9)
WILD TYPE
(n = 9)
200
180
160
140
120
100
80
60
40
20
0
SERUM IFN GAMMA LEVELS (pg/ml)

Fig. 7

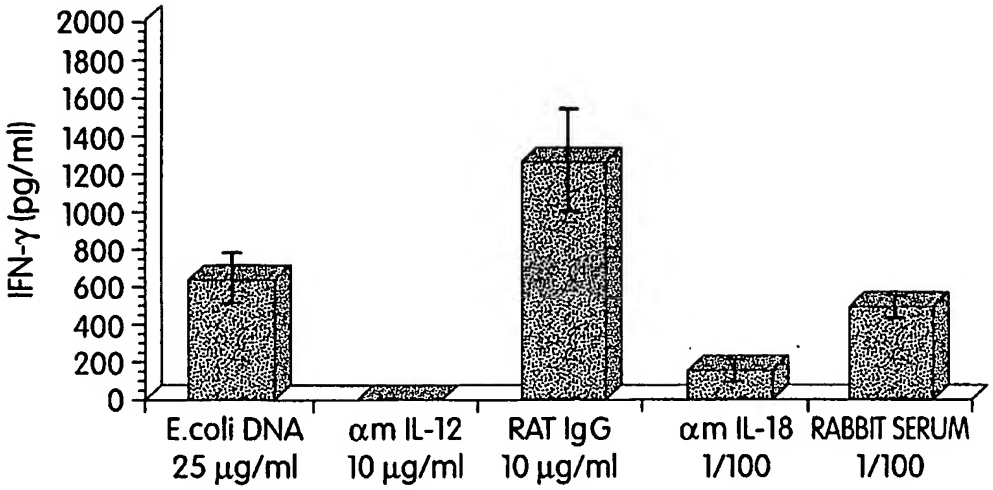

IFN-γ (pg/ml)
2000
1800
1600
1400
1200
1000
800
600
400
200
0
E.coli DNA 25 µg/ml
αm IL-12 10 µg/ml
RAT IgG 10 µg/ml
αm IL-18 1/100
RABBIT SERUM 1/100

Fig. 8A

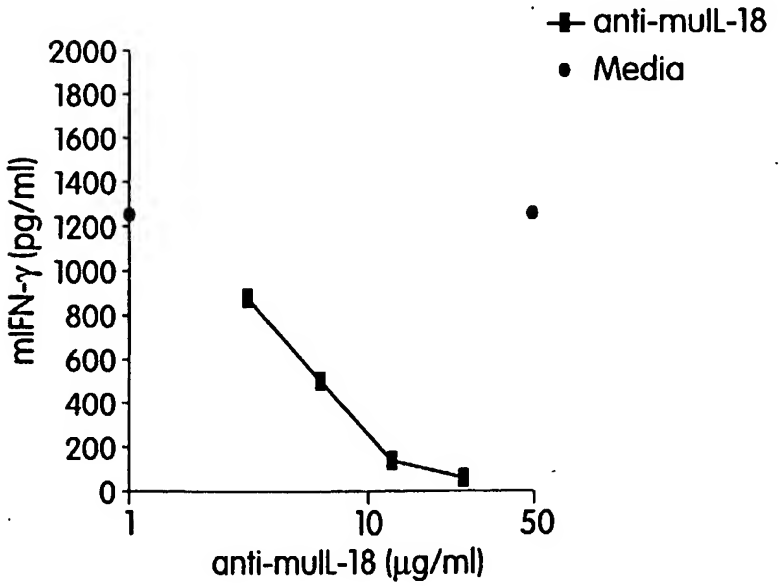

anti-mulL-18
Media
mIFN-γ (pg/ml)
2000
1800
1600
1400
1200
1000
800
600
400
200
0
1
10
50
anti-mulL-18 (µg/ml)

Fig. 8B

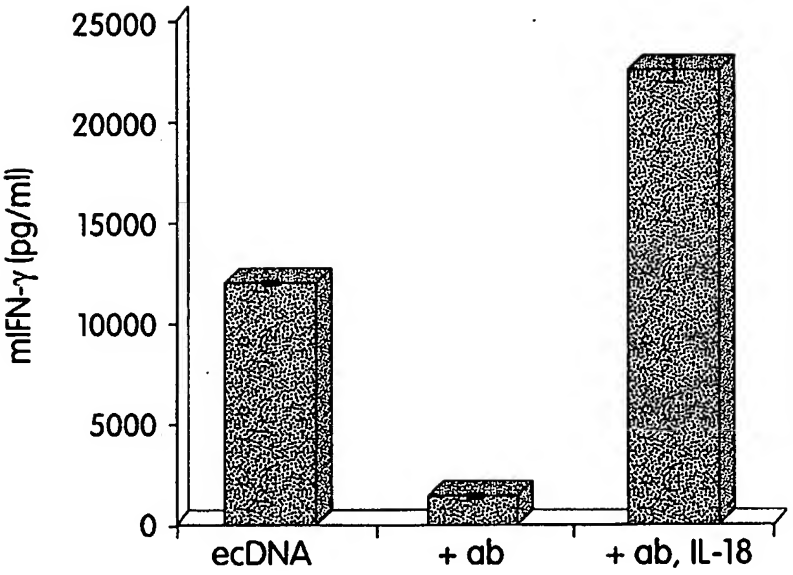


Fig. 8C

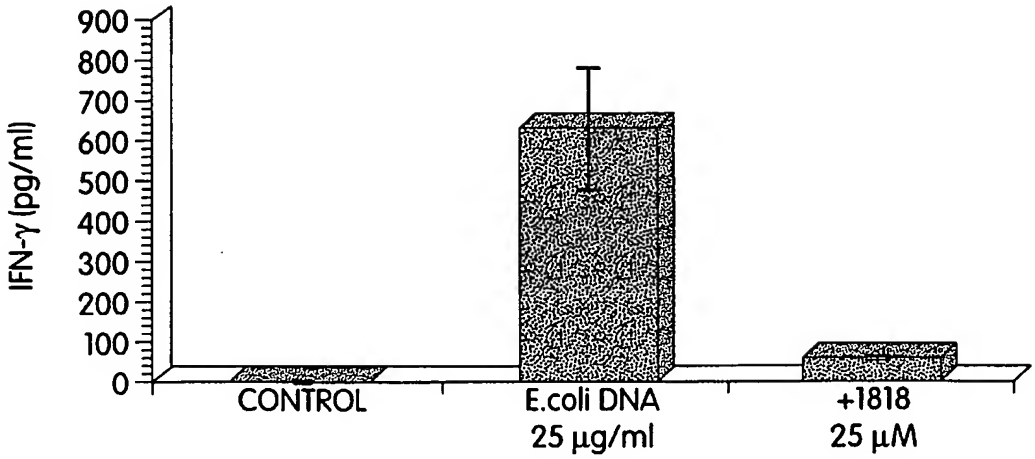

900
800
700
600
500
400
300
200
100
0
IFN-γ (pg/ml)
CONTROL
E.coli DNA
25 µg/ml
+1818
25 µM

Fig. 9A

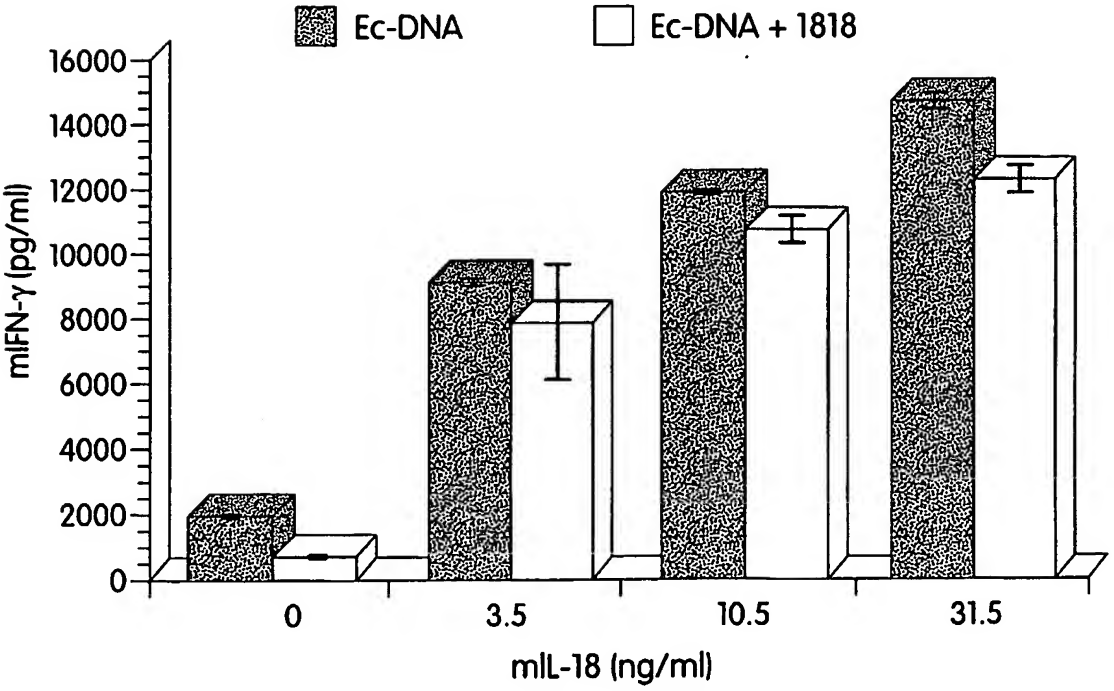

Ec-DNA
Ec-DNA + 1818
16000
14000
12000
10000
8000
6000
4000
2000
0
mIFN-γ (pg/ml)
0
3.5
10.5
31.5
mIL-18 (ng/ml)

Fig. 9B

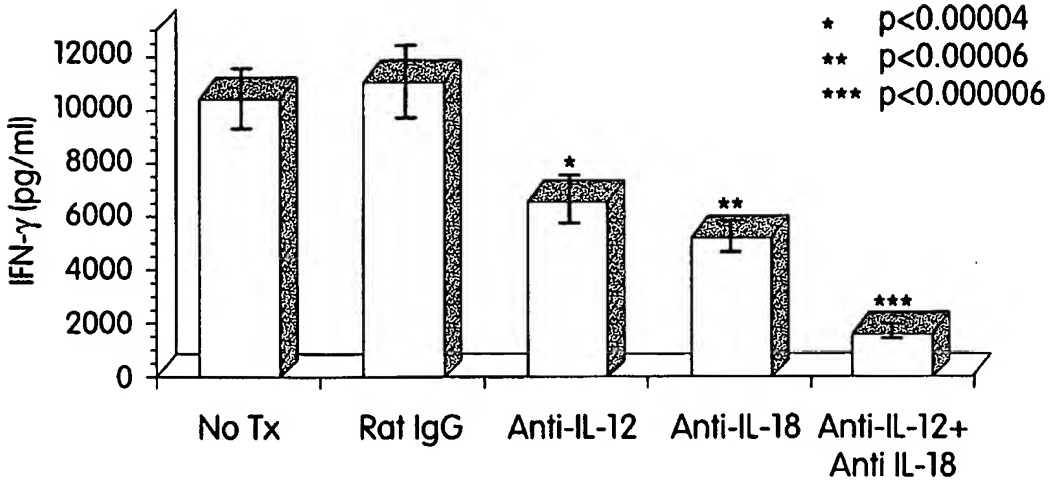


Fig. 10A

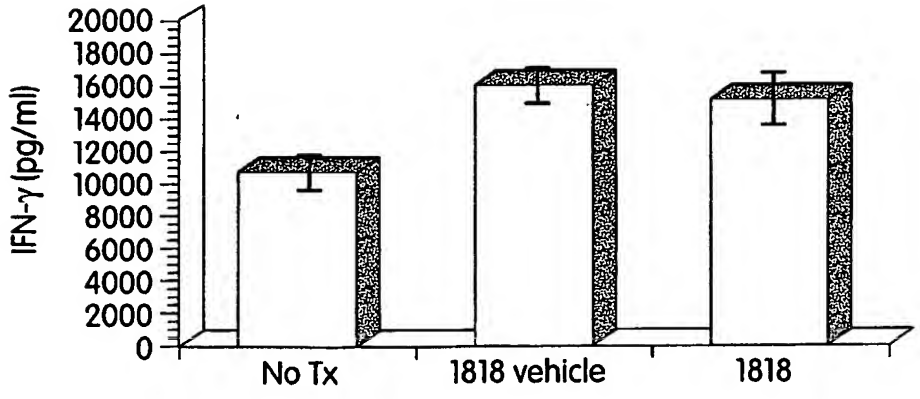


Fig. 10B

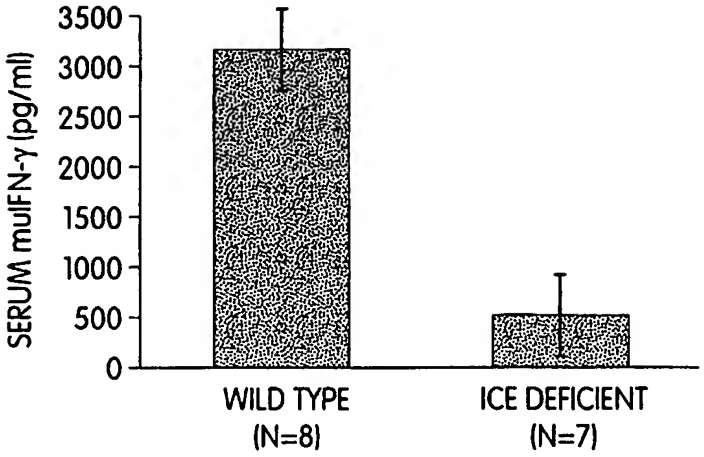


Fig. 11A

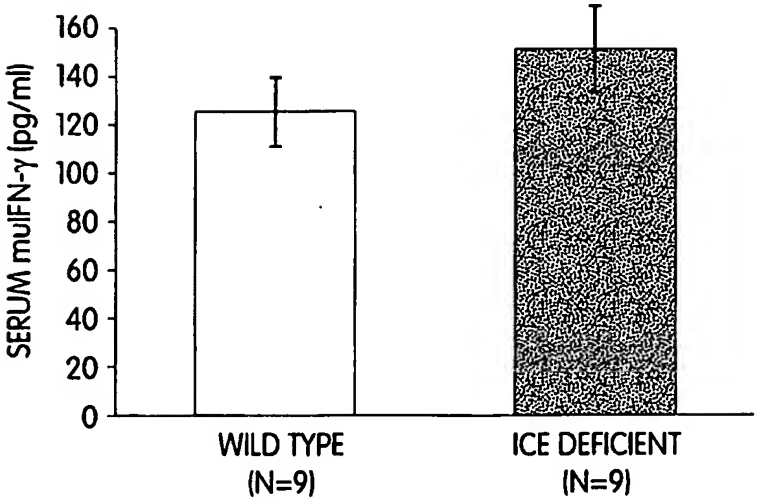


Fig. 11B

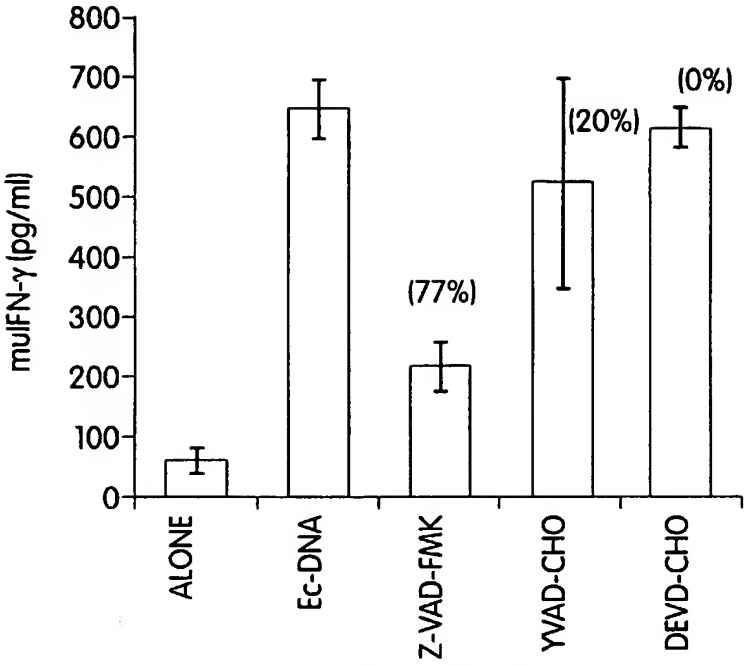


Fig. 12A

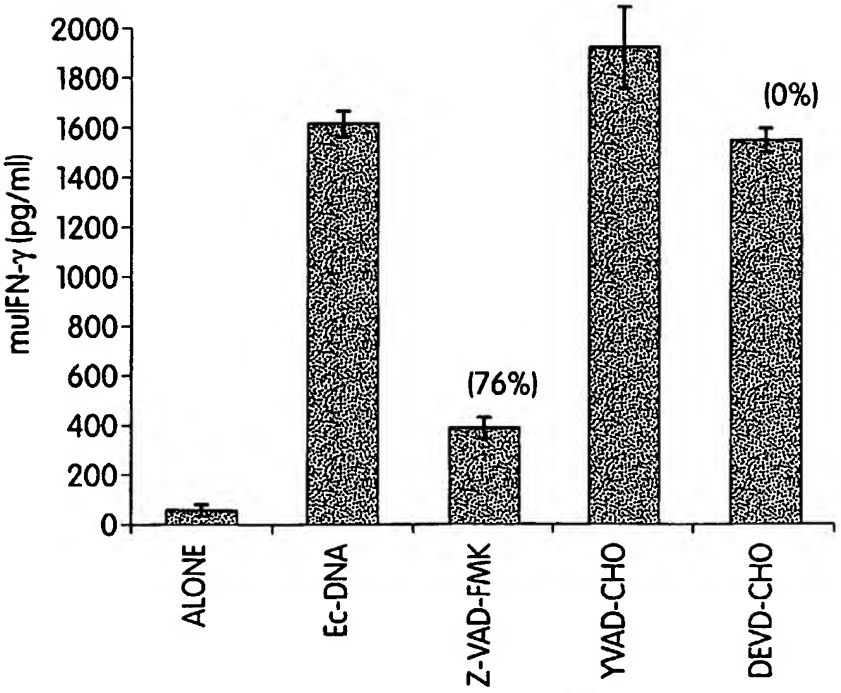


Fig. 12B

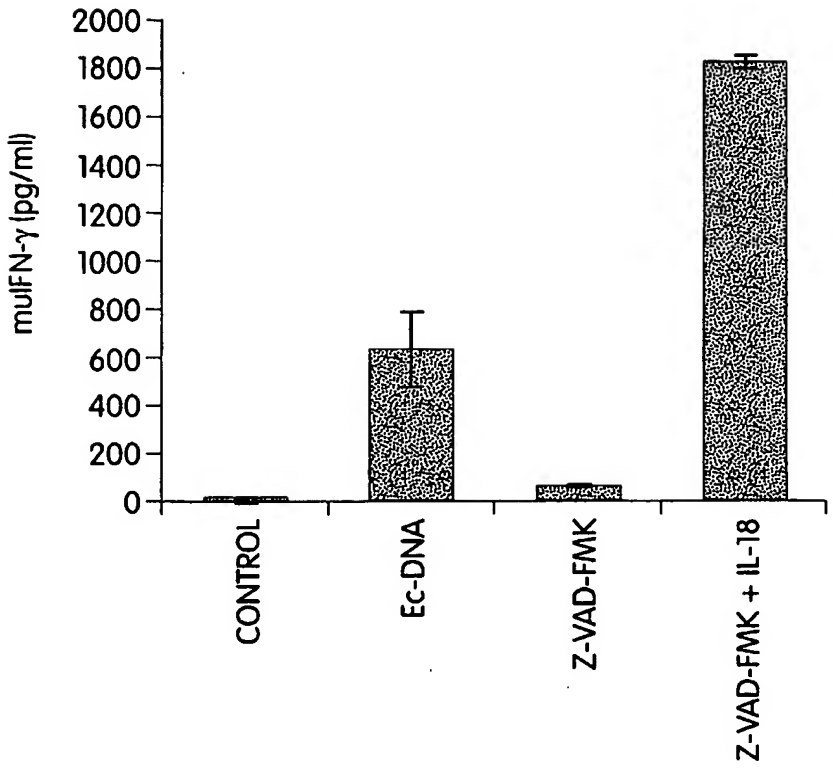

muIFN-γ (pg/ml)
2000
1800
1600
1400
1200
1000
800
600
400
200
0
CONTROL
Ec-DNA
Z-VAD-FMK
Z-VAD-FMK + IL-18

Fig. 12C